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(54) Title: NUCLEIC ACID MODULE CODING FOR ALPHA GLUCOSIDASE, PLANTS THAT SYNTHESIZE MODIFIED STARCH, METHODS FOR THE PRODUCTION AND USE OF SAID PLANTS, AND MODIFIED STARCH

(54) Bezeichnung: NUKLEINSÄUREMOLEKÜLE KODIEREND FÜR EINE α-GLUKOSIDASE, PFLANZEN, DIE EINE MODIFIZIERTE STÄRKE SYNTHETISIEREN, VERFAHREN ZUR HERSTELLUNG DER PFLANZEN, IHRE VERWENDUNG SOWIE DIE MODIFIZIERTE STÄRKE

(57) Abstract

The present invention relates to nucleic acid molecules coding for a protein with the activity of an alpha-glucosidase from a potato.The invention also relates to methods for the production of transgenic plant cells and plants synthesizing modified starch. The invention further relates to vectors and host cells containing the inventive nucleic acid modules, plant cells and plants obtained according to the inventive methods, starch synthesized by the inventive plant cells and methods for the production of said starch.

(57) Zusammenfassung

Die vorliegende Erfindung betrifft Nukleinsäuremoleküle, die ein Protein mit der Aktivität einer α-Glukosidase aus Kartoffel kodieren sowie Verfahren zur Herstellung transgener Pflanzenzellen und Pflanzen, die eine modifizierte Stärke synthetisieren. Des weiteren betrifft die vorliegende Erfindung Vektoren und Wirtszellen, welche die erfindungsgemäßen Nukleinsäuremoleküle enthalten, die aus den erfindungsgemäßen Verfahren hervorgehenden Pflanzenzellen und Pflanzen, die von den erfindungsgemäßen Pflanzenzellen und Pflanzen synthetisierte Stärke sowie Verfahren zur Herstellung dieser Stärke.

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Nucleic acid molecules encoding an α-glucosidase, plants which synthesize a modified starch, the generation of the plants, their use, and the modified starch

The present invention relates to nucleic acid molecules which encode a protein with the activity of a potato α-glucosidase, and to processes for the generation of transgenic plant cells and plants which synthesize a modified starch. Moreover, the present invention relates to vectors and host cells comprising the nucleic acid molecules according to the invention, to the plant cells and plants originating from the processes according to the invention, to the starch synthesized by the plant cells and plants according to the invention, and to processes for the production of this starch.

Taking into consideration the increasing importance attached to plant constituents as renewable raw materials, biotechnology research attempts to adapt plant-based raw materials to the needs of the processing industry. To allow renewable raw materials to be used in as many fields of application as possible, it is therefore necessary to provide a multiplicity of substances.

Besides oils, fats and proteins, polysaccharides constitute important renewable raw materials from plants. Besides cellulose, starch, which is one of the most important storage substances in higher plants, occupies a central position amongst the polysaccharides. Besides maize, rice and wheat, potatoes play an important role, in particular in starch production.

The polysaccharide starch is a polymer of chemically uniform units, the glucose molecules. However, it is a highly complex mixture of different forms of molecules which differ with regard to their degree of polymerization and the occurrence of branchings of the glucose chains. Starch therefore constitutes no uniform raw material. In particular, we differentiate between amylose starch, an essentially unbranched polymer of α-1,4-glycosidically linked glucose molecules, and amylopectin starch, which, in turn, constitutes a complex mixture of differently branched glucose chains. The branchings are generated by the occurrence of additional α-1,6-glycosidic linkages. In typical plants used for starch production such as, for example, maize or potatoes, the starch synthesized consists of approx. 25% amylose starch and approx. 75% amylopectin starch.

The molecular structure of starch, which is determined to a great extent by the degree of branching, the amylose/amylopectin ratio, the average length and distribution of the side chains, and the presence of phosphate groups, is decisive for important functional properties of starch or its aqueous solutions. Examples of functional properties which must be mentioned in this context are solubility, the retrogradation behavior, the film-forming properties, the viscosity, the color stability, the gelatinization properties, and binding and adhesive properties. The starch granule size may also be of importance for various uses. Also, the generation of high-amylose starches is of particular interest for certain applications. Furthermore, a modified starch present in plant cells can advantageously modify the behavior of the plant cell under certain conditions. For example, it is feasible to reduce starch breakdown during the storage of starch-containing organs, such as, for example, seeds or tubers, prior to their further processing, for example for extracting the starch. It is furthermore of interest to prepared modified starches which lead to plant cells or plant organs containing this starch being better suited to processing, for example in the production of foods such as popcorn or cornflakes from maize, or the production of French fries, chips or potato powder from potatoes. Of particular interest in this context is an improvement of the starches with regard to reduced cold sweetening, i.e. a reduced liberation of reducing sugars (in particular glucose) upon prolonged storage at low temperatures. Potatoes especially are frequently stored at temperatures from 4 to 8°C in order to minimize starch breakdown during storage. The reducing sugars liberated during this process, in particular glucose, result in undesired browning reactions (so-called Maillard reactions) in the production of French fries or crisps.

The starch which can be isolated from plants is frequently adapted to particular industrial purposes with the aid of chemical modifications which, as a rule, require time and money. It seems therefore desirable to find possibilities of generating plants which synthesize starch whose properties already meet the specific demands of the processing industry and thus combine economical and ecological advantages.

One possibility of providing such plants is, in addition to plant breeding measures, the directed genetic modification of the starch metabolism of

starch-producing plants by recombinant methods. However, a prerequisite therefor is the identification and characterization of the enzymes which participate in starch synthesis modification and starch breakdown (starch metabolism) and the isolation of the corresponding DNA sequences which encode these enzymes.

The biochemical synthetic pathways which lead to the synthesis of starch are essentially known. In plant cells, starch synthesis takes place in the plastids. In photosynthetically active tissues, these plastids are the chloroplasts, in photosynthetically inactive, starch-storing tissue the amyloplasts.

Important enzymes which are involved in starch metabolism are, for example, the branching enzymes, ADP glucose pyrophosphorylases, granule-bound starch synthases, soluble starch synthases, debranching enzymes, disproportioning enzymes, plastid starch phosphorylases, the R1 enzymes (R1 proteins), amylases or glucosidases.

It is an object of the present invention to provide other, or alternative, recombinant approaches for modifying the starch metabolism in starch-synthesizing plants (for example rye, barley, oats, maize, wheat, sorghum and millet, sago, rice, peas, marrowfat peas, cassava, potatoes, tomatoes, oilseed rape, soybeans, hemp, flax, sunflowers, cowpeas, mung beans, beans, bananas or arrowroot) suitable nucleic acid molecules by means of which plant cells can be transformed, thus allowing the synthesis of modified, advantageous starch species.

Such modified starch species exhibit, for example, modifications regarding their degree of branching, the amylose/amylopectin ratio, the phosphate content, the starch granule size and/or the average length and distribution of the side chains (i.e. side chain structure).

It is a further object of the invention to provide methods which allow the generation of transgenic plants which synthesize a modified starch species.

Surprisingly, transgenic plants which have been transformed with the nucleic acid molecules according to the invention synthesize a starch whose physicochemical properties and/or whose side chain structure is

modified in the particular manner so that the abovementioned objects are achieved by providing the use forms specified in the claims.

The invention therefore relates to a nucleic acid molecule encoding a protein with the function of a potato α-glucosidase, selected from the group consisting of

a) nucleic acid molecules which encode a protein which encompasses the amino acid sequence stated under Seq ID NO. 2 or its derivatives or parts,

b) nucleic acid molecules which encompass the nucleotide sequence shown under Seq ID No. 1 or its derivatives or parts, or a corresponding ribonucleotide sequence;

c) nucleic acid molecules which hybridize with, or are complementary to, preferably which hybridize specifically with, the nucleic acid molecules stated under a) or b), and

d) nucleic acid molecules whose nucleotide sequence deviates from the sequence of the nucleic acid molecules stated under a), b) or c) owing to the degeneracy of the genetic code.

Accordingly, the present invention relates to a nucleic acid molecule which encodes an α-glucosidase and which comprises an amino acid sequence of Seq No. 2 or its derivatives or parts in accordance with the cDNA insert of the plasmid (DSM No. 12347). The abovementioned α-glucosidase according to the invention is involved in the starch metabolism of potatoes and is directly or indirectly involved in starch biosynthesis.

The term "derivative" with regard to the α-glucosidase protein (or its polypeptide, amino acid sequence) of the invention encompasses, for the purposes of the present invention, a polypeptide which is derived from Seq ID No. 2 and which comprises at least 163 amino acid residues, preferably at least 227, in particular at least 293 and very especially preferably approximately 309-322 amino acid residues which are selected from the group of the amino acid residues consisting of

18 H, 25 R, 34 G, 37 H, 38 G, 39 V, 41 L, 42 L, 44 S, 45 N, 46 G, 47 M, 48 D, 51 Y, 53 G, 55 R, 56 I, 58 Y, 60 V, 61 I, 62 G, 63 G, 65 I, 66 D, 67 L, 68 Y, 70 F, 71 A, 72 G, 75 P, 78 V, 81 Q, 83 T, 86 I, 87 G, 88 R, 89 P, 90 A, 92 M, 93 P, 94 Y, 95 W, 97 F, 98 G, 99 F, 101 Q, 102 C, 103 R, 105 G, 106 Y, 115 V, 116 V, 119 Y, 120 A, 124 I, 125 P, 126 L, 127 E, 128 V, 129 M, 130 W, 131 T, 132 D, 133 I, 134 D, 135 Y, 136 M, 137 D, 140 K, 141 D, 142 F, 143 T, 144 L, 145 D, 146 P, 147 V, 149 F, 150 P, 157 F, 161 L, 162 H, 164 N, 166 Q, 168 Y, 169 V, 171 I, 173 D, 174 P, 175 G, 176 I, 182 Y, 184 T, 187 R, 188 G, 189 M, 193 V, 194 F 196 K, 197 R, 201 P, 202 Y, 204 G, 206 V, 207 W, 208 P, 209 G, 211 V, 212 Y, 214 P, 215 D, 216 F, 217 L, 219 P, 224 F, 225 W, 228 E, 229 I, 232 F, 237 P, 239 D, 240 G, 242 W, 244 D, 245 M, 246 N, 247 E, 249 S, 250 N, 251 F, 252 I, 254 S, 260 S, 263 D, 265 P, 266 P, 267 Y, 268 K, 269 I, 270 N, 271 N, 272 S, 273 G, 277 P, 278 I, 282 T, 284 P, 286 T, 289 H, 291 G, 295 E, 296 Y, 299 H, 300 N, 301 L, 303 G, 305 L, 306 E, 310 T, 313 A, 322 P, 323 F, 325 L, 327 R, 328 S, 329 T, 330 F, 333 S, 334 G, 336 Y, 337 T, 339 H, 340 W, 341 T, 342 G, 343 D, 344 N, 345 A, 346 A, 348 W, 350 D, 351 L, 353 Y, 354 S, 355 I, 356 P, 359 L, 361 F, 362 G, 363 L, 364 F, 365 G, 367 P, 368 M, 370 G, 371 A, 372 D, 373 I, 374 C, 375 G, 376 F, 380 T, 381 T, 382 E, 383 E, 384 L, 385 C, 387 R, 388 W, 389 I, 390 Q, 391 L, 392 G, 393 A, 394 F, 395 Y, 396 P, 397 F, 399 R, 400 D, 401 H, 402 S, 406 T, 409 Q, 410 E, 411 L, 412 Y, 414 W, 416 S, 417 V, 418 A, 421 A, 424 V, 425 L, 426 G, 427 L, 428 R, 431 L, 432 L, 433 P, 436 Y, 438 L, 439 M, 440 Y, 442 A, 446 G, 448 P, 449 I, 450 A, 451 R, 452 P, 453 L, 455 F, 457 F, 458 P, 460 D, 463 T, 466 I, 469 Q, 470 F, 471 L, 473 G, 477 M, 479 S, 480 P, 482 L, 485 G, 489 V, 491 A, 492 Y, 494 P, 496 G, 497 N, 498 W, 501 L, 504 Y, 508 V, 513 G, 518 L, 521 P, 523 D, 524 H, 526 N, 527 V, 528 H, 531 E, 532 G, 534 I, 537 M, 538 Q, 539 G, 541 A, 543 T, 544 T, 547 A, 550 T, 554 L, 555 L, 556 V, 557 V, 559 S, 566 G, 567 E, 568 L, 569 F, 571 D, 579 G, 583 G, 585 W, 586 T, 588 V, 590 F, 603 S, 605 V, 606 V, 611 A, 620 K, 622 T, 625 G, 635 Y, 658 F, 664 S, 669 L, 671 G, 674 F

and 678 L of Seq ID No. 2 and which comprises at least approximately 1-69, preferably at least 139, in particular at least 194, more preferably at least 249 and very especially preferably approximately 263-274 amino acid

residues which are selected from the group of the amino acid residues consisting of

1 P, 2 K, 3 L, 4 R, 5 P, 6 R, 7 V, 8 H, 9 P, 10 S, 11 Q, 12 H, 13 H, 14 P, 15 I, 16 Q, 17 L, 19 R, 20 P, 21 P, 22 A, 23 L, 24 H, 27 Y, 28 S, 29 F, 30 R, 31 Y, 32 F, 35 V, 36 S, 43 S, 49 I, 50 V, 57 S, 64 L, 84 Q, 91 A, 109 I, 110 D, 112 V, 114 L, 118 S, 122 S, 152 E, 153 R, 154 V, 155 I, 156 F, 158 L, 159 R, 163 Q, 165 D, 172 V, 178 I, 180 N, 183 D, 186 R, 198 D, 199 N, 200 M, 203 Q, 205 V, 210 N, 221 T, 222 E, 223 V, 226 R, 230 E, 231 K, 236 V, 238 F, 243 L, 259 S, 262 F, 275 H, 280 Y, 281 R, 288 T, 293 T, 294 M, 311 Y, 312 S, 316 N, 317 V, 326 V, 331 L, 335 R, 338 S, 360 S, 378 S, 404 K, 408 P, 413 S, 420 A, 422 K, 430 Q, 437 M, 444 I, 445 K, 447 T, 461 A, 464 F, 465 D, 468 T, 478 I, 481 I, 487 T, 510 L, 511 N, 512 Q, 516 M, 536 V, 548 Q, 549 R, 551 A, 553 K, 558 L, 560 S, 561 S, 562 K, 570 V, 573 D, 574 D, 577 Q, 580 R, 581 E, 584 R, 591 N, 592 S, 593 N, 594 I, 595 I, 598 K, 599 I, 601 V, 602 K, 609 R, 612 L, 613 D, 615 G, 616 L, 618 L, 619 E, 623 L, 630 R, 631 G, 632 L, 634 S, 637 L, 638 V, 639 G, 641 H, 642 Q, 643 Q, 644 G, 645 N, 646 T, 647 T, 648 M, 649 K, 650 E, 651 S, 652 L, 653 K, 654 Q, 656 G, 657 Q, 659 V, 660 T, 661 M, 666 M, 668 I, 670 I, 679 Y, 680 I, 681 I, 682 T, 693 H, 700 R, 703 G, 705 H, 706 G, 707 V, 709 L, 710 L, 712 S, 713 N, 714 G, 715 M, 716 D, 718 Y, 720 G, 721 R, 722 I, 724 Y, 726 V, 727 I, 728 G, 729 G, 730 I, 731 D, 732 L, 733 Y, 734 F, 735 A, 736 G, 739 P, 742 V, 743 Q, 745 T, 747 I, 748 G, 749 R, 750 P, 751 A, 753 M, 754 P, 755 Y, 756 W, 757 F, 758 G, 759 F, 761 Q, 762 C, 763 R, 764 G, 765 Y, 768 V, 769 V, 771 Y, 772 A, 775 I, 776 P, 777 L, 778 E, 779 V, 780 M, 781 W, 782 T, 783 D, 784 I, 785 D, 786 Y, 787 M, 788 D, 789 K, 790 D, 791 F, 792 T, 793 L, 794 D, 795 P, 796 V, 798 F, 799 P, 804 F, 806 L, 807 H, 808 N, 810 Q, 812 Y, 813 V, 814 I, 816 D, 817 P, 818 G, 819 I, 821 Y, 822 T, 824 R, 825 G, 826 M, 828 V, 829 F, 831 K

and 832 R (here identified by the single-letter code for amino acids) of Seq ID No. 2.

The term "part" with regard to the α-glucosidase protein (polypeptide, amino acid sequence) according to the invention encompasses, for the purposes of the present invention, a poly- or oligopeptide composed of at least approximately 10-50, preferably at least 100, more preferably at least

200, especially preferably at least 400 and most preferably approximately 550-675 of the amino acid residues of the α-glucosidase encoded by the nucleic acid molecule according to the invention or its derivatives.

The present invention furthermore relates to a nucleic acid molecule which comprises a nucleic acid molecule of Seq ID No. 1 in accordance with the cDNA insert of the plasmid DSM No. 12347 deposited at the DSZM on 07.24.1998, or its derivatives or parts, in particular of the coding region or its derivatives or parts.

The term "derivative" with regard to the nucleic acid molecule (nucleotide sequence or polynucleotide) according to the invention encompasses, for the purposes of this invention, a polynucleotide which comprises at least 478 nucleotides, preferably at least 668, in particular at least 860, and very especially preferably approximately 907-945 nucleotides selected from the group consisting of

4 A, 6 A, 12 A, 13 C , 17 G, 25 C, 32 A, 34 C, 38 A, 45 T, 47 A, 49 C, 51 T, 56 G, 62 C, 65 C, 68 T, 73 C, 76 G, 77 G, 78 A, 79 T, 97 G, 100 G, 101 G, 106 A, 108 T, 109 C, 110 A, 111 T, 112 G, 113 G, 114 G, 115 G, 116 T, 119 T, 122 T, 125 T, 127 A, 130 A, 131 G, 132 C, 133 A, 134 A, 135 T, 136 G, 137 G, 139 A, 140 T, 141 G, 142 G, 143 A, 144 T, 146 T, 151 T, 152 A, 153 T, 157 G, 158 G, 161 A, 162 T, 164 G, 166 A, 167 T, 169 A, 171 T, 172 T,

173 A, 174 C, 175 A, 176 A, 178 G, 179 T, 181 A, 182 T, 183 T, 184 G, 185 G, 187 G, 188 G, 191 T, 193 A, 194 T, 195 T, 196 G, 197 A, 200 T, 202 T, 203 A, 206 T, 208 T, 209 T, 211 G, 212 C, 214 G, 215 G, 216 A, 217 C, 221 C, 223 C, 224 C, 226 G, 232 G, 233 T, 236 T, 237 G, 239 A, 241 C, 242 A, 243 G, 244 T, 247 A, 248 C, 249 T, 254 T, 256 A, 257 T, 259 G, 260 G, 263 G, 265 C, 266 C, 268 G, 269 C, 272 C, 274 A, 275 T, 276 G, 277 C, 278 C, 280 T, 281 A, 283 T, 284 G, 285 G, 289 T, 290 T, 292 G, 293 G, 297 T, 298 C, 299 A, 301 C, 302 A, 304 T, 305 G, 308 G, 310 T, 313 G, 314 G, 316 T, 317 A, 323 A, 324 T, 326 T, 331 G, 332 A, 335 T, 338 A, 343 G, 344 T, 346 G, 347 T, 349 G, 355 T, 356 A, 357 T, 358 G, 359 C, 360 A, 362 A, 365 C, 366 T, 370 A, 371 T, 373 C, 374 C, 377 T, 379 G, 380 A, 382 G, 383 T, 385 A, 386 T, 387 G, 388 T, 389 G, 390 G, 391 A, 392 C, 394 G, 395 A, 397 A, 398 T, 399 T, 400 G, 401 A, 402 T, 403 T, 404 A, 406 A, 407 T, 408 G, 409 G, 410 A, 411 T, 412 G, 415 T, 418 A, 419 A, 421 G, 422 A, 424 T, 425 T, 426 C, 427 A, 428 C, 431 T, 433 G, 434 A, 436 C, 437 C, 439 G, 440 T, 443 A, 445 T, 446 T, 448 C, 449 C, 454 G, 455 A, 459 G, 461 T, 469 T, 470 T, 471 T, 473 T, 478 A, 481 C, 482 T, 484 C, 485 A, 489 G, 490 A, 491 A, 492 T, 493 G, 496 C, 497 A, 499 A, 501 A, 502 T, 503 A, 505 G, 506 T, 511 A, 512 T, 515 T, 517 G, 518 A, 519 T, 520 C, 521 C, 523 G, 524 G, 526 A, 527 T, 536 A, 538 A, 542 C, 544 T, 545 A, 546 T, 547 G, 550 A, 551 C, 553 T, 556 A, 559 A, 560 G, 562 G, 563 G, 565 A, 566 T, 567 G, 569 A, 570 A, 575 A, 576 T, 577 G, 578 T, 580 T, 581 T, 584 T, 586 A, 587 A, 590 G, 593 A, 594 T, 597 T, 601 C, 602 C, 604 T, 605 A, 607 C, 610 G, 611 G, 616 G, 617 T, 619 T, 620 G, 621 G, 622 C, 623 C, 625 G, 626 G, 631 G, 632 T, 634 T, 635 A, 637 T, 640 C, 641 C, 643 G, 644 A, 646 T, 647 T, 650 T, 653 A, 655 C, 656 C, 659 C, 660 T, 662 C, 663 T, 670 T, 671 T, 673 T, 674 G, 675 G, 680 A, 682 G, 683 A, 685 A, 686 T, 689 A, 690 G, 694 T, 695 T, 697 C, 701 A, 704 T, 707 T, 709 C, 710 C, 713 T, 715 G, 716 A, 717 T, 718 G, 719 G, 722 T, 724 T, 725 G, 726 G, 728 T, 730 G, 731 A, 733 A,

734 T, 735 G, 736 A, 737 A, 739 G, 740 A, 745 T, 746 C, 748 A, 749 A, 751 T, 752 T, 754 A, 755 T, 758 C, 759 T, 760 T, 761 C, 764 C, 766 C, 773 C, 778 T, 779 C, 780 T, 782 C, 785 T, 787 G, 788 A, 791 A, 792 T, 793 C, 794 C, 796 C, 797 C, 799 T, 800 A, 802 A, 803 A, 805 A, 806 T, 808 A, 809 A, 811 A, 812 A, 814 T, 815 C, 817 G, 818 G, 820 G, 829 C, 830 C, 832 A, 833 T, 835 A, 841 A, 844 A, 845 C, 848 T, 850 C, 851 C, 854 C, 856 A, 857 C, 860 C, 862 A, 865 C, 866 A, 868 T, 870 T, 871 G, 872 G, 875 A, 883 G, 884 A, 886 T, 887 A, 890 A, 891 T, 892 G, 895 C, 896 A, 897 T, 898 A, 899 A, 902 T, 904 T, 906 T, 907 G, 908 G, 914 T, 916 G, 917 A, 918 A, 920 C, 921 T, 924 A, 925 G, 927 C, 928 A, 929 C, 937 G, 938 C, 941 T, 944 T, 953 C, 958 A, 962 G, 964 C, 965 C, 967 T, 968 T, 971 T, 974 T, 979 A, 980 G, 982 T, 983 C, 985 A, 986 C, 988 T, 989 T, 990 T, 995 G, 997 T, 998 C, 1000 G, 1001 G, 1003 A, 1006 T, 1007 A, 1008 C, 1009 A, 1010 C, 1013 C, 1015 C, 1016 A, 1018 T, 1019 G, 1020 G, 1021 A, 1022 C, 1024 G, 1025 G, 1027 G, 1028 A, 1029 T, 1030 A, 1031 A, 1032 T, 1033 G, 1034 C, 1035 T, 1036 G, 1037 C, 1039 A, 1042 T, 1043 G, 1044 G, 1046 A, 1048 G, 1049 A, 1052 T, 1057 T, 1058 A, 1059 C, 1060 T, 1061 C, 1063 A, 1064 T, 1066 C, 1067 C, 1072 A, 1073 T, 1076 T, 1080 C, 1081 T, 1082 T, 1083 T, 1084 G, 1085 G, 1088 T, 1090 T, 1091 T, 1092 T, 1093 G, 1094 G, 1096 A, 1097 T, 1099 C, 1100 C, 1102 A, 1103 T, 1104 G, 1106 T, 1108 G, 1109 G, 1111 G, 1112 C, 1114 G, 1115 A, 1116 T, 1117 A, 1118 T, 1120 T, 1121 G, 1123 G, 1124 G, 1125 T, 1126 T, 1127 T, 1138 A, 1139 C, 1141 A, 1142 C, 1144 G, 1145 A, 1147 G, 1148 A, 1151 T, 1153 T, 1154 G, 1157 G, 1159 C, 1160 G, 1162 T, 1163 G, 1164 G, 1165 A, 1166 T, 1168 C, 1169 A, 1170 G, 1171 C, 1172 T, 1174 G, 1175 G, 1177 G, 1178 C, 1180 T, 1181 T, 1183 T, 1184 A, 1186 C, 1187 C, 1189 T, 1190 T, 1193 C, 1195 A, 1196 G, 1198 G, 1199 A, 1201 C, 1202 A, 1204 T, 1205 C, 1208 C, 1213 G, 1216 A, 1217 C, 1220 C, 1225 C, 1226 A, 1228 G, 1229 A, 1231 C, 1232 T, 1234 T, 1235 A, 1240 T, 1241 G, 1242 G, 1243 G, 1244 A, 1246 T, 1247 C, 1249 G, 1250 T,

1252 G, 1253 C, 1254 T, 1256 C, 1259 C, 1261 G, 1262 C, 1264 A, 1267 A, 1270 G, 1271 T, 1274 T, 1276 G, 1277 G, 1279 C, 1280 T, 1281 C, 1283 G, 1292 T, 1294 C, 1295 T, 1297 C, 1298 C, 1301 A, 1306 T, 1307 A, 1309 A, 1313 T, 1315 A, 1316 T, 1317 G, 1318 T, 1319 A, 1321 G, 1322 A, 1324 G, 1325 C, 1328 A, 1331 T, 1333 A, 1336 G, 1337 G, 1339 A, 1342 C, 1343 C, 1345 A, 1346 T, 1348 G, 1349 C, 1351 C, 1352 G, 1354 C, 1355 C, 1357 C, 1358 T, 1360 T, 1362 C, 1363 T, 1364 T, 1367 C, 1369 T, 1370 T, 1372 C, 1373 C, 1376 A, 1378 G, 1379 A, 1387 A, 1388 C, 1390 T, 1393 G, 1396 A, 1397 T, 1403 C, 1405 C, 1406 A, 1407 G, 1408 T, 1409 T, 1412 T, 1415 T, 1417 G, 1418 G, 1420 A, 1422 A, 1424 G, 1427 T, 1429 A, 1430 T, 1431 G, 1433 T, 1435 T, 1436 C, 1438 C, 1439 C, 1444 C, 1445 T, 1448 A, 1450 C, 1453 G, 1454 G, 1456 G, 1463 C, 1465 G, 1466 T, 1470 T, 1471 G, 1472 C, 1474 T, 1475 A, 1477 T, 1480 C, 1481 C, 1486 G, 1487 G, 1488 A, 1489 A, 1490 A, 1492 T, 1493 G, 1494 G, 1496 T, 1501 C, 1502 T, 1504 T, 1508 A, 1510 T, 1511 A, 1514 C, 1520 C, 1522 G, 1523 T, 1527 T, 1533 T, 1537 G, 1538 G, 1540 A, 1544 A, 1547 T, 1549 A, 1552 C, 1559 C, 1561 C, 1562 C, 1565 C, 1567 G, 1568 A, 1569 T, 1570 C, 1571 A, 1575 T, 1577 A, 1578 A, 1579 G, 1580 T, 1582 C, 1583 A, 1586 T, 1588 C, 1591 G, 1592 A, 1593 A, 1594 G, 1595 G, 1597 A, 1600 A, 1601 T, 1604 T, 1605 G, 1606 G, 1609 A, 1610 T, 1611 G, 1612 C, 1613 A, 1614 A, 1615 G, 1616 G, 1619 A, 1621 G, 1622 C, 1625 T, 1626 G, 1627 A, 1628 C, 1630 A, 1631 C, 1636 G, 1639 G, 1640 C, 1645 A, 1648 A, 1649 C, 1652 C, 1654 T, 1658 A, 1660 C, 1661 T, 1664 T, 1666 G, 1667 T, 1669 G, 1670 T, 1675 A, 1676 G, 1689 C, 1690 A, 1692 C, 1696 G, 1697 G, 1699 G, 1700 A, 1703 T, 1705 T, 1706 T, 1709 T, 1711 G, 1712 A, 1715 A, 1717 G, 1724 A, 1727 T, 1732 A, 1733 T, 1735 G, 1736 G, 1745 G, 1746 A, 1747 G, 1748 G, 1750 A, 1753 T, 1754 G, 1755 G, 1756 A, 1757 C, 1760 T, 1762 G, 1763 T, 1765 A, 1768 T, 1769 T, 1775 G, 1776 C, 1781 T, 1783 A, 1789 A, 1796 T, 1802 T, 1807 T, 1808 C, 1809 A, 1810 G, 1811 A, 1813 G, 1814 T, 1816 G, 1817 T, 1828 T, 1830 T, 1831 G, 1832 C, 1836 G, 1845 A, 1846 T, 1848 G, 1851 C, 1853 T, 1855 G, 1858 A, 1859 A, 1862 T, 1864 A, 1865 C, 1868 T, 1871 T, 1873 G, 1874 G, 1876 T, 1877 T, 1881 A, 1890 A, 1894 T, 1897 A, 1901 G, 1908 G, 1925 A, 1929 A, 1945 A, 1953 T, 1958 A, 1966 G, 1972 T, 1973 T, 1976 T, 1984 G, 1988 T, 1990 T, 1991 C, 1997 T, 2006 T, 2009 T, 2011 G, 2012 G, 2020 T, 2021 T, 2024 A, 2027 T

and 2033 T of Seq ID No. 1 and which furthermore comprises at least approximately 1-93 nucleotides, preferably at least 187, in particular 261, more preferably at least 336 and very especially preferably approximately 354-369 nucleotides selected from the group consisting of

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1 C, 10 A, 16 A, 19 G, 21 T, 23 A, 24 C, 26 C, 30 A, 33 A, 36 C, 39 T, 43 A, 48 G, 52 C, 53 A, 54 C, 57 T, 58 C, 59 C, 60 G, 63 G, 64 G, 66 G, 67 C, 69 C, 70 C, 71 A, 72 C, 74 G, 75 G, 80 A, 81 C, 86 T, 88 C, 89 G, 91 T, 93 C, 94 T, 96 C, 99 C, 102 A, 103 G, 104 T, 105 T, 107 G, 123 T, 128 G, 138 C, 145 A, 149 T, 156 G, 170 G, 189 G, 190 T, 192 A, 199 T, 201 G, 264 T, 271 G, 279 A, 291 C, 294 A, 309 G, 325 A, 327 T, 328 G, 333 T, 334 G, 340 C, 341 T, 342 G, 348 G, 354 T, 363 G, 364 T, 375 G, 384 T, 420 G, 429 A, 432 C, 438 A, 444 C, 456 G, 457 C, 458 G, 460 G, 462 A, 464 T, 465 T, 467 T, 468 T, 472 C, 476 G, 477 G, 480 G, 486 T, 487 C, 494 A, 507 A, 513 A, 514 G, 516 A, 522 A, 525 A, 534 C, 537 C, 540 T, 543 A, 549 C, 552 C, 557 G, 558 G, 564 C, 573 A, 592 G, 595 A, 596 A, 599 T, 603 C, 608 A, 609 A, 612 G, 614 T, 627 G, 636 T, 639 T, 651 A, 661 A, 665 A, 667 G, 668 T, 669 A, 678 A, 687 T, 688 G, 692 A, 706 G, 708 A, 727 C, 744 G, 771 A, 774 A, 775 T, 776 C, 783 C, 784 T, 798 C, 807 A, 813 C, 819 C, 825 C, 834 C, 838 T, 842 G, 843 A, 855 C, 858 T, 863 C, 864 A, 878 C, 879 A, 881 T, 882 G, 885 G, 888 T, 903 T, 912 A, 931 T, 934 A, 935 G, 940 T, 949 G, 954 T, 957 T, 976 G, 977 T, 987 T, 991 C, 1002 C, 1004 G, 1012 T, 1038 T, 1041 C, 1062 C, 1068 T, 1079 G, 1087 T, 1095 A, 1132 A, 1140 T, 1161 C, 1167 T, 1179 A, 1188 A, 1203 C, 1206 T, 1210 A, 1211 A, 1212 G, 1215 C, 1223 C, 1224 C, 1239 T, 1258 G, 1263 C, 1265 A, 1275 T, 1278 G, 1287 T, 1288 C, 1291 T,

1293 A, 1296 T, 1305 T, 1310 T, 1311 G, 1312 C, 1314 T, 1330 A, 1338 G, 1340 C, 1341 T, 1344 C, 1347 T, 1353 A, 1356 C, 1386 G, 1389 A, 1391 T, 1402 A, 1416 C, 1432 A, 1441 A, 1443 A, 1446 T, 1455 A, 1459 A, 1460 C, 1461 C, 1467 T, 1497 T, 1500 C, 1503 C, 1518 C, 1521 T, 1528 T, 1530 G, 1531 A, 1534 C, 1535 A, 1546 A, 1557 C, 1563 A, 1566 A, 1575 A, 1581 A, 1590 T, 1596 G, 1602 A, 1603 T, 1607 T, 1608 C, 1632 A, 1641 T, 1643 A, 1644 G, 1650 T, 1651 G, 1653 A, 1657 A, 1659 A, 1665 T, 1668 C, 1672 C, 1678 A, 1680 C, 1681 A, 1683 C, 1684 A, 1695 A, 1698 A, 1704 A, 1708 G, 1718 A, 1719 C, 1738 A, 1743 G, 1749 G, 1751 G, 1752 G, 1758 G, 1761 A, 1772 A, 1773 C, 1774 A, 1784 T, 1785 T, 1788 C, 1791 T, 1792 A, 1795 A, 1800 G, 1801 G, 1803 T, 1805 A, 1812 G, 1815 T, 1825 C, 1834 C, 1837 G, 1842 A, 1843 G, 1847 T, 1852 C, 1857 A, 1869 A, 1875 A, 1878 T, 1884 T, 1886 T, 1891 G, 1895 T, 1896 G, 1902 C, 1903 T, 1904 A, 1905 T, 1906 G, 1909 C, 1911 T, 1913 T, 1914 T, 1915 G, 1918 T, 1919 C, 1920 A, 1922 A, 1923 C, 1924 C, 1932 G, 1936 A, 1940 C, 1948 G, 1949 A, 1955 T, 1957 A, 1959 G, 1960 C, 1962 G, 1964 G, 1969 C, 1975 G, 1979 C, 1981 A, 1986 A, 1989 C, 1995 G, 1996 A, 2001 A, 2002 A, 2005 T, 2007 G, 2008 A, 2035 T, 2038 A, 2040 C, 2042 T, 2044 A, 2045 C, 2046 T, 2047 T **and 2048 A of Seq ID No. 1.**

In the numbering of the positions of the individual elements of the nucleotide or amino acid sequences according to the invention of Seq ID No. 1 or Seq ID No. 2, which has been stated above explicitly, derivatives of said sequences according to the invention are also to be understood as meaning those sequences in which the numbering of the individual sequence elements may deviate from those of the sequences ID No. 1 or No. 2 according to the invention. What is decisive here is significant agreement of at least one sequence section ("part") with the sequence according to the invention. Such agreements can be determined in a simple manner using general expert knowledge, for example by making use of suitable computer programs, for example by carrying out a sequence comparison of the sequence according to the invention with a sequence in question to be compared (so-called sequence alignment). Such computer programs, which, for example, are commercially available (for example Omiga®, Version 1.1.3. by Oxford Molecular Ltd., Oxford, UK) and which in some cases are also an integral component of sequence databases (for example EMBL, GenBank), identify, for example, the best-possible agreement of

identical, or, if appropriate, chemical equivalent, sequence elements and take into consideration in particular the existence of insertions and/or deletions which may lead to a shift of individual sequence elements or of sequence sections and which can thus affect numbering of the sequence elements or sequence sections.

With regard to the nucleic acid molecule according to the invention which encodes an α-glucosidase, the term "derivative" furthermore encompasses a nucleic molecule which deviates from Seq ID No. 1 by addition, deletion, insertion or recombination of one or more nucleotides and which meets the conditions as defined above.

With regard to the nucleic acid molecule according to the invention which encodes an α-glucosidase, the term "derivative" furthermore comprises a complementary or inverted-complementary sequence (polynucleotide) of the nucleic acid molecule according to the invention or of derivatives or parts thereof.

The term "part", which refers to the nucleic acid molecule according to the present invention which encodes an α-glucosidase, encompasses a poly- or oligonucleotide composed of at least approximately 15-35, preferably at least approximately 36-100, in particular at least 200, more preferably at last 400, especially preferably at least 800 and most preferably approximately 1400-1700 of the nucleotides of a nucleic acid molecule according to the invention which encodes an α-glucosidase, or their derivatives.

In a preferred embodiment of the present invention, the terms "derivative" and/or "part" according to the present invention encompass a polynucleotide, or a poly- or oligopeptide as defined above, which shows functional and/or structural equivalence of the α-glucosidase gene obtained from potato (i.e. of the nucleic acid molecule which encodes the α-glucosidase) or α-glucosidase polypeptide. The term "functional and/or structural equivalence" generally means the same, an equivalent or similar function of the inventive molecule in question, if appropriate especially biological function.

The invention furthermore relates to a recombinant nucleic acid molecule comprising a) a nucleotide sequence encoding a protein with the function of an α-glucosidase, preferably from potato, or parts of said nucleotide sequence, and b) one or more nucleotide sequences which encode a protein selected from amongst group A, composed of proteins with the function of branching enzymes, ADP glucose pyrophosphorylases, granule-bound starch synthases, soluble starch synthases, debranching enzymes, disproportioning enzymes, plastid starch phosphorylases, R1 enzymes, amylases, glucosidases, parts of nucleotide sequences encoding proteins selected from amongst group A and nucleic acid molecules which hybridize with one of said nucleotide sequences or parts thereof, preferably a deoxyribonucleic acid or ribonucleic acid molecule, especially preferably a cDNA molecule. Especially preferred is a nucleic acid molecule which specifically hybridizes with one of said nucleotide sequences or parts thereof.

The nucleotide sequence according to the invention encoding a protein with the function of a potato α-glucosidase is depicted by Seq ID No. 1, the protein encoded by the nucleotide sequence by Seq ID No. 2.

Seq. ID No. 1:

cgaatacgaataaccgacgctaaccatcaacgatgggaagtgccggaagaaattctccac

cgtccaccaccgccgtcgccgccgtcaacctccaactcctcatcagaaaaccactcccca

attaccctctctaacccaaactcagacctagagttcacccttcacaacaccatcccattc

agcttcaccgtccgccggcgctccaccggggatactcttttcgatacttcgccggagtta

gtcatggggttttgcttctgagtagcaatggcatggatattgtgtatacgggtgatagga

ttagttacaaggtgattggagggttaattgatttgtatttctttgccggaccttcgccgg

aaatggtggtggatcagtatactcagcttattggtcgtcctgctgctatgccatattggt

ctttcggatttcaccaatgccggtggggttacaagaatattgatgatgttgaactggtag

tggatagttatgcaaagtctagaataccgctggaggttatgtggactgatattgattaca

tggatggttttaaggacttcacactcgatccagttaacttcccactggagcgggtaattt

tttttctcaggaagcttcatcagaatgatcagaaatatgtactaatagtagatccaggaa

ttagcatcaacaatacatatgacacctataggagaggcatggaagcagatgtcttcataa

aacgcgataatatgccctaccaaggggttgtttggccagggaatgtttattatcctgatt

ttctaaatccagctactgaagtattttggagaaatgaaattgagaagttccaggatctcg

tacctttgatggcctgtggcttgacatgaatgaattgtcaaacttcataacttcccctc

ctacaccatcatctacctttgatgatcctccctacaagataaacaactctggcgatcact

tgcccatcaattatagaacagttccagccacttctacacattttggtgatacaatggagt

ataatgtccataacctttatggattacttgaatctagagccacttatagtgcattggtta

atgtcactggtaaaaggccattcattcttgtaagatcaacttttcttggctctggcagat

acacgtcacattggactggagataatgctgctacctggaacgatttggcatactccattc

ctactatcttgagctttggattgtttggaattccaatggttggagctgatatatgtggtt

tttcaagtaacactactgaagagctttgccgccgctggattcagcttggagcattctatc

catttgcaagagaccactctgctaaggacacaaccccccaagagctctatagttgggatt

cagttgctgcagcagccaagaaagtccttgggctccgatatcagttacttccatacttt

atatgcttatgtacgaggcacatataaaagggactcccattgcacgacccctcttcttct

ctttccctcaagatgccaagacatttgatatcagcacacagttccttctcggtaaaggtg

tcatgatctcacctatacttaagcaaggagcaacctctgttgatgcatatttccctgctg

gaaactggtttgacctcttcaattactctcgctctgtgagtttgaatcaaggaacatata

tgacacttgacgcaccaccagatcatataaatgtacatgttcgtgaagggaacatattgg

tcatgcaaggggaagcaatgacaacacaagctgctcagaggactgcattcaaactccttg
tcgtgctgagcagcagcaaaaacagcacaggagaactatttgtggacgatgacgatgagg
tgcagatgggaagagagggagggaggtggacgctagttaagtttaacagcaatatcattg
gcaataaaattgtggttaaatcagaggttgtgaatggacgatatgcgctggatcaaggat
tggtccttgaaaaggtgacattattgggatttgaaaatgtgagaggattgaagagctatg
agcttgttggatcacaccagcaagggaacacaacaatgaaggaaagtcttaagcagagtg
gacagtttgttactatggaaatctcagggatgtcaatattgatagggaaagagttcaaat
tggagctatacatcattacttaacaaatgaattaagttatatacgcttgttgtatgaaat
tttctttcatttatcaatgcagtttaatttatgataaaaaaaaaaaaaaaaaa

Seq. ID No. 2:

PKLRPRVHPSQHHPIQLHRPPALHRGYSFRYFAGVSHGVLLLSSNGMDIVYTGDRISYKV
IGGLIDLYFFAGPSPEMVVDQYTQLIGRPAAMPYWSFGFHQCRWGYKNIDDVELVVDSYA
KSRIPLEVMWTDIDYMDGFKDFTLDPVNFPLERVIFFLRKLHQNDQKYVLIVDPGISINN
TYDTYRRGMEADVFIKRDNMPYQGVVWPGNVYYPDFLNPATEVFWRNEIEKFQDLVPFDG
LWLDMNELSNFITSPPTPSSTFDDPPYKINNSGDHLPINYRTVPATSTHFGDTMEYNVHN
LYGLLESRATYSALVNVTGKRPFILVRSTFLGSGRYTSHWTGDNAATWNDLAYSIPTILS
FGLFGIPMVGADICGFSSNTTEELCRRWIQLGAFYPFARDHSAKDTTPQELYSWDSVAAA
AKKVLGLRYQLLPYFYMLMYEAHIKGTPIARPLFFSFPQDAKTFDISTQFLLGKGVMISP
ILKQGATSVDAYFPAGNWFDLFNYSRSVSLNQGTYMTLDAPPDHINVHVREGNILVMQGE
AMTTQAAQRTAFKLLVVLSSSKNSTGELFVDDDDEVQMGREGGRWTLVKFNSNIIGNKIV
VKSEVVNGRYALDQGLVLEKVTLLGFENVRGLKSYELVGSHQQGNTTMKESLKQSGQFVT
MEISGMSILIGKEFKLELYIIT

The α-glucosidase nucleotide sequence according to the invention shows relatively little sequence homology with known α-glucosidase-encoding molecules (Taylor et al., 1998, Plant J. 13: 419-424; Sugimoto et al., 1997, Plant Mol. Biol. 33, 765-768; EMBL Datenbank-Einträge: U22450, P10253, D86624). The amino acid sequence differs markedly from the α-glucosidases described in the prior art, in particular in the 5' region, as can be seen from a sequence alignment with Seq ID No. 2.

Nucleotide sequences which encode a protein of group A and which are suitable according to the invention have been described, for example, for soluble (types I, II, III or IV) or granule-bound starch synthase isoforms in Hergersberg, 1988, Ph.D. thesis, University of Cologne; Abel, 1995, Ph.D. thesis, FU Berlin; Abel et al., 1996, Plant Journal 10(6):981-991; Visser et al., 1989, Plant Sci. 64:185-192; van der Leij et al., 1991, Mol. Gen. Genet. 228:240-248; EP-A-0779363; WO 92/11376; WO 96/15248; WO 97/26362;

WO 97/44472; WO 97/45545; Delrue et al., 1992, J. Bacteriol. 174: 3612-3620; Baba et al., 1993, Plant Physiol. 103:565-573; Dry et al., 1992, The Plant Journal 2,2: 193-202 or else in the EMBL database entries X74160; X58453; X88789; X 94400; for branching enzyme isoforms (branching enzymes I, Iia or IIb), debranching enzyme isoforms (debranching enzyme, isoamylases, pullulanases, R1 enzymes) or disproportioning enzyme isoforms, for example, described in WO 92/14827; WO 95/07335; WO 95/09922; WO 96/19581; WO 97/22703; WO 97/32985; WO 97/42328; Takaha et al., 1993, J. Biol. Chem. 268: 1391-1396 or else in the EMBL database entry X83969, and those for ADP glucose pyrophosphorylases and plastid starch phosphorylase isoforms, for example, described in EP-A-0368506; EP-A-0455316; WO 94/28146; DE 19653176.4; WO 97/11188; Brisson et al., 1989, The Plant Cell 1:559-566; Buchner et al., 1996, Planta 199:64-73; Camirand et al., 1989, Plant Physiol. 89(4 Suppl.) 61; Bhatt & Knowler, J. Exp. Botany 41 (Suppl.) 5-7; Lin et al., 1991, Plant Physiol. 95: 1250-1253; Sonnewald et al., 1995, Plant Mol. Biol. 27:567-576; DDBJ No. D23280; Lorberth et al., 1998, Nature Biotechnology 16:473-477.

The nucleotide sequences to be employed suitably in accordance with the invention are of pro- or eukaryotic origin, preferably of bacterial, fungal or plant origin.

The term "parts of nucleotide sequences" denotes, for the purposes of the present invention, parts of the nucleotide sequences to be used in accordance with the invention which are at least 15 bp, preferably at least 150 bp, especially preferably at least 500 bp in length, but which do not exceed a length of 5000 bp, preferably 2500 bp.

The term "hybridization" means, for the purposes of the present invention, hybridization under conventional hybridization conditions, preferably under stringent conditions, as are described, for example, in Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd Ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

A "specific hybridization" especially preferably takes place under the following highly stringent conditions:

Hybridization buffer: 2 x SSC; 10 x Denhardt solution (Fikoll 400 + PEG + BSA; ratio 1:1:1); 0.1% SDS; 5 mM EDTA; 50 mM Na_2HPO_4; 250 µg/ml herring sperm DNA; 50 µg/ml tRNA; or 0.25 M sodium phosphate buffer pH 7.2; 1 mM EDTA; 7% SDS at a

Hybridization temperature:	T = 55 to 68°C,
Wash buffer:	0.2 x SSC; 0.1% SDS and
Wash temperature:	T = 40 to 68°C.

The molecules which hybridize with the nucleic acid molecules according to the invention also encompass fragments, derivatives and allelic variants of the nucleic acid molecules according to the invention. Fragments are to be understood as meaning parts of the nucleic acid molecules which are long enough to encode a functionally active part of the proteins described. The term derivative means in this context that the sequences of these molecules differ from the sequences of the nucleic acid molecules according to the invention in one or more positions and exhibit a high degree of homology to these sequences. Homology means a sequence identity of at least 60%, preferably over 70% and especially preferably over 85%. The deviations relative to the nucleic acid molecules according to the invention may have originated by means of deletions, substitutions, insertions or recombinations.

Homology furthermore means that functional and/or structural equivalence exists between the nucleic acid molecules in question or the proteins encoded by them. The nucleic acid molecules which are homologous to the molecules according to the invention and which constitute derivatives of these molecules are, as a rule, variations of these molecules which constitute modifications which exert the same biological function. They may be naturally occurring variations, for example sequences from other plant species, or mutations, it being possible for these mutations to have occurred naturally or to have been introduced by directed mutagenesis. The variations may further be synthetic sequences. The allelic variants may be naturally occurring variants or else synthetic variants or variants generated by recombinant DNA technology.

The nucleic acid molecules according to the invention may be DNA molecules, in particular cDNA or genomic molecules. The nucleic acid

molecules according to the invention may furthermore be RNA molecules. The nucleic acid molecules according to the invention or parts thereof can have been obtained, for example, from natural sources or generated by means of recombinant technology or by synthesis.

To express the nucleic acid molecules according to the invention in sense or antisense orientation in plant cells, they are linked to regulatory DNA elements which ensure transcription in plant cells. These include, in particular, promoters. In general, any promoter which is active in plant cells is suitable for expression. The promoter may have been chosen in such a way that expression is constitutive or only in a particular tissue, at a particular point in time of plant development or at a point in time determined by external factors which can be, for example, chemically or biologically inducible. Relative to the transformed plant, the promoter - and also the nucleotide sequence - can be homologous or heterologous. Examples of suitable promoters are the cauliflower mosaic virus 35S RNA promoter for constitutive expression, the patatin gene promoter B33 (Rocha-Sosa et al., 1989, EMBO J. 8:23-29) for tuber-specific expression in potatoes or a promoter which ensures expression only in photosynthetically active tissues, for example the ST-LS1 promoter (Stockhaus et al., 1987, Proc. Natl. Acad. Sci. USA 84: 7943-7947; Stockhaus et al., 1989, EMBO J. 8: 2445-2451) or, for endosperm-specific expression, the wheat HMG promoter or promoters from maize zein genes.

A termination sequence which terminates the nucleic acid molecule according to the invention may serve to correctly end transcription and to add to the transcript a poly-A tail, which is considered to have a function in stabilizing the transcripts. Such elements have been described in the literature (cf. Gielen et al., 1989, EMBO J. 8:23-29) and are exchangeable as desired.

The nucleic acid molecules according to the invention can be used for generating transgenic plant cells and plants which show an increase or reduction in the activity of α-glucosidase or in the activity of α-glucosidase and at least one further enzyme of starch metabolism. To this end, the nucleic acid molecules according to the invention are introduced into suitable vectors, provided with the regulatory nucleic acid sequences which

are necessary for efficient transcription in plant cells, and introduced into plant cells. On the one hand, there is the possibility of using the nucleic acid molecules according to the invention for inhibiting the synthesis of the endogenous α-glucosidase or the endogenous α-glucosidase and at least one further protein of group A in the cells. This can be achieved with the aid of antisense constructs, in-vivo mutagenesis, a cosuppression effect which occurs, or with the aid of suitably constructed ribozymes. On the other hand, the nucleic acid molecules according to the invention can be used for expressing α-glucosidase or α-glucosidase and at least one further protein of group A in the cells of transgenic plants and thus lead to an increased activity in the cells of the enzymes which have been expressed in each case.

In addition, there exists the possibility of using the nucleic acid molecules according to the invention for inhibiting the synthesis of the endogenous α-glucosidase and the overexpression of at least one further protein of group A in the cells.

Finally, the nucleic acid molecules according to the invention can also be used for expressing α-glucosidase and inhibiting at least one further protein of group A in the cells of transgenic plants. The two last-mentioned embodiments of the invention thus lead, in the cells, to a simultaneous inhibition and increase in the activities of the enzymes which are inhibited or expressed, respectively.

The invention furthermore relates to a vector comprising a nucleic acid molecule according to the invention.

The term "vector" encompasses plasmids, cosmids, viruses, bacteriophages and other vectors conventionally used in genetic engineering which contain the nucleic acid molecules according to the invention and which are suitable for transforming cells. Such vectors are preferably suitable for transforming plant cells. Especially preferably, they permit integration of the nucleic acid molecules according to the invention, if appropriate together with flanking regulatory regions, into the genome of the plant cell. Examples are binary vectors, such as pBinAR or pBinB33, which can be employed in agrobacteria-mediated gene transfer.

In a preferred embodiment, the vector according to the invention is distinguished by the fact that the nucleotide sequence encoding a protein with the function of an α-glucosidase or parts thereof is present in sense or antisense orientation.

In a further preferred embodiment, the vector according to the invention is distinguished by the fact that the nucleotide sequence which encodes one or more proteins selected from amongst group A or parts thereof is present in sense or antisense orientation.

In yet a further preferred embodiment, the vector according to the invention is distinguished by the fact that the nucleotide sequence which encodes a plurality of proteins selected from group A or parts thereof is present partly in sense and partly in antisense orientation.

Very especially preferably, the vector according to the invention is linked to regulatory elements which ensure expression in a prokaryotic or eukaryotic cell, i.e., for example, transcription and synthesis of an RNA which, if the nucleotide sequence is present in sense orientation, is translatable.

In addition, it is possible to introduce, by means of customary techniques of molecular biology (see, for example, Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2nd Ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbour, NY), various mutations into the DNA sequences according to the invention, which leads to the synthesis of proteins with biological properties which may have been modified. On the one hand, it is possible to generate deletion mutants in which sequences are generated, by progressive deletions from the 5' or the 3' end of the coding DNA sequences which lead to the synthesis of analogously truncated proteins. For example, such deletions at the 5' end of the DNA sequence allow the targeted production of enzymes which, due to the removal of the relevant transit or signal sequences, are no longer localized in their original (homologous) compartment, but in the cytosol, or which, due to the addition of other signal sequences, are localized in one or more other (heterologous) compartments.

On the other hand, it is also feasible to introduce point mutations in positions where an altered amino acid sequence affects, for example, the enzyme activity or the regulation of the enzyme. Thus, it is possible, for example, to generate mutants which have an altered K_M or k_{cat} value or which are no longer subject to the regulatory mechanisms normally present in the cell via allosteric regulation or covalent modification.

For the purposes of recombination manipulation in prokaryotic cells, the DNA sequences according to the invention or parts of these sequences can be introduced into plasmids which permit mutagenesis or an altered sequence by the recombination of DNA sequences. Base exchanges may be performed or natural or synthetic sequences may be added, with the aid of standard methods in molecular biology (cf. Sambrook et al., 1989, loc. cit.). To link the DNA fragments to each other, adapters or linkers may be attached to the fragments. Furthermore, manipulations which provide suitable restriction cleavage sites or which remove excessive DNA or restriction cleavage sites which are no longer needed may be employed. Where insertions, deletions or substitutions are suitable, *in-vitro* mutagenesis, primer repair, restriction or ligation may be used. The analytical methods which are generally employed are sequence analysis, restriction analysis and, if appropriate, other methods of biochemistry and molecular biology.

The invention furthermore relates to a host cell, in particular prokaryotic or eukaryotic cells, preferably bacterial or plant cells (for example from E. coli, Agrobacterium, Solananceae, Poideae, rye, barley, oats, maize, wheat, sorghum and millet, sago, rice, peas, marrowfat peas, cassava, potatoes, tomatoes, oilseed rape, soybeans, hemp, flax, sunflowers, cowpeas, mung beans, beans, bananas or arrowroot) which contains a nucleic acid molecule according to the invention or a vector according to the invention or which is derived from a cell which has been transformed with a nucleic acid molecule according to the invention or a vector according to the invention.

The invention furthermore relates to a host cell, in particular prokaryotic or eukaryotic cells, preferably bacterial or plant cells (for example of E. coli, Agrobacterium, Solanaceae, Poideae, rye, barley, oats, maize, wheat,

sorghum and millet, sago, rice, peas, marrowfat peas, cassava, potatoes, tomatoes, oilseed rape, soybeans, hemp, flax, sunflowers, cowpeas, mung beans, beans, bananas or arrowroot) which contains, in addition to a recombinant nucleic acid molecule encoding a protein with the function of a β-amylase, one or more further recombinant nucleic acid molecules which encode a protein selected from group A or their parts or nucleotide sequences hybridizing with these nucleic acid molecules.

In addition to using the nucleic acid molecules according to the invention, the host cells according to the invention may, if appropriate, also be generated by successive transformation (so-called supertransformation), by employing individual nucleotide sequences or vectors comprising nucleotide sequences which encode a protein with the function of branching enzymes, ADP glucose pyrophosphorylases, granule-bound starch synthases, soluble starch synthases I, II, III or IV, debranching enzymes, disproportioning enzymes, plastid starch phosphorylases, R1 enzymes, amylases, glucosidases, parts thereof, and nucleic acid molecules which hybridizes with one of said nucleotide sequences or their parts, in a plurality of successive cell transformations. A further embodiment of the present invention relates to a method of generating a transgenic plant cell which synthesizes a modified starch, which comprises integrating a nucleic acid molecule according to the invention or a vector according to the invention into the genome of a plant cell.

Providing the nucleic acid molecules according to the invention makes it possible to engage in the start metabolism of plants, with the aid of recombinant methods, and to alter it in such a way that the result is the synthesis of a modified starch which is altered relative to the starch synthesized in the wild-type plant with regard to, for example, structure, water content, protein content, lipid content, fiber content, ash/phosphate content, amylase/amylopectin ratio, molecular mass distribution, degree of branching, granule size, granule shape and crystallization, or else in its physicochemical properties such as the viscoelasticity, the sorptive characteristics, gelatinization temperature, viscosity, thickening capacity, solubility, gel structure, transparency, thermal stability, shear stability, stability to acids, tendency to undergo retrogradation, gelling, freeze-thaw stability, complex formation, iodine binding, film formation, adhesion power, enzyme stability,

digestibility or reactivity. There is also the possibility of increasing the yield in suitably genetically modified plants by increasing the activity of proteins which are involved in starch metabolism, for example by overexpressing suitable nucleic acid molecules, or by providing mutants which are no longer subject to the cell's regulatory mechanisms and/or which exhibit different temperature dependencies relating to their activity. A particularly pronounced increase in yield may be the result of increasing the activity of one or more proteins which are involved in the starch metabolism in specific cells of the starch-storing tissue of transformed plants such as, for example, in the tuber in the case of potatoes or in the endosperm of maize or wheat. The economic importance and the advantages of these possibilities of engaging in the starch metabolism are obvious.

When expressing the nucleic acid molecules according to the invention in plants it is possible, in principle, for the protein synthesized to be localized in any desired compartment of the plant cell. To achieve localization in a particular compartment (cytosol, vacuole, apoplast, plastids, mitochondria), the transit or signal sequence which ensures localization must, if necessary, be deleted (removed) and the remaining coding region must, if necessary, be linked to DNA sequences which ensure localization in the compartment in question. Such sequences are known (see, for example, Braun et al., EMBO J. 11 (1992), 3219-3227; Wolter et al., Proc. Natl. Acad. Sci. USA 85 (1988), 846-850; Sonnewald et al., Plant J. 1 (1991), 95-106).

The generation of plant cells with a reduced activity of a protein involved in the starch metabolism can be achieved, for example, by expressing a suitable antisense RNA, a sense RNA for achieving a cosuppression effect, in-vivo mutagenesis or by expressing a suitably constructed ribozyme which specifically cleaves transcripts which encode one of the proteins involved in starch metabolism, using a nucleic acid molecule according to the invention, preferably by expressing an antisense transcript.

To this end, it is possible to use, firstly, a DNA molecule which encompasses all of the sequence which encodes a protein involved in starch metabolism including any flanking sequences, as well as DNA molecules which only encompass parts of the coding sequence, these

parts having a minimum length of 15 bp, preferably of at least 100-500 bp, and in particular over 500 bp. As a rule, DNA molecules are used which are shorter than 5000 bp, preferably shorter than 2500 bp.

It is also possible to use DNA sequences which exhibit a high degree of homology to the sequences of the DNA molecules according to the invention, but are not fully identical with them. The minimum homology should exceed approx. 65%. The use of sequences with a homology of 75% and in particular 80% is to be preferred.

The expression of ribozymes for reducing the activity of specific proteins in cells is known to the skilled worker and described, for example, in EP-B1 0 321 201. The expression of ribozymes in plant cells were described, for example, in Feyter et al. (Mol. Gen. Genet. 250 (1996), 329-338).

Furthermore, the reduction of the proteins involved in the starch metabolism in the plant cells according to the invention can also be achieved by so-called "in-vivo mutagenesis", where an RNA-DNA hybrid oligonucleotide ("chimeroplast") is introduced into cells by cell transformation (Kipp P.B. et al., Poster Session at the "5th International Congress of Plant Molecular Biology, 21-27, September 1997, Singapore; R.A. Dixon and C.J. Arntzen, Meeting report on "Metabolic Engineering in Transgenic Plants", Keystone Symposia, Copper Mountain, CO, USA, TIBTECH 15 (1997), 441-447; international patent application WO 95/15972; Kren et al., Hepatology 25 (1997), 1462-1468; Cole-Strauss et al., Science 273 (1996), 1386-1389).

Part of the DNA component of the RNA-DNA oligonucleotide used for this purpose is homologous to a nucleic acid sequence of an endogenous protein, but exhibits a mutation in comparison with the nucleic acid sequence of the endogenous protein or comprises a heterologous region enclosed by the homologous regions.

Base pairing of the homologous regions of the RNA-DNA oligonucleotide and of the endogenous nucleic acid molecule followed by homologous recombination allows the mutation or heterologous region contained in the DNA component of the RNA-DNA oligonucleotide to be transferred into the

genome of a plant cell. This leads to a reduced activity of the protein involved in the starch metabolism.

As an alternative, the enzyme activities which are involved in the starch metabolism can be reduced in the plant cells by a cosuppression effect. This method is known to the skilled worker and is described, for example, by Jorgensen (Trends Biotechnol. 8 (1990), 340-344), Niebel et al., (Curr. Top. Microbiol. Immunol. 197 (1995), 91-103), Flavell et al. (Curr. Top. Microbiol. Immunol. 197 (1995), 43-46), Palaqui and Vaucheret (Plant. Mol. Biol. 29 (1995), 149-159), Vaucheret et al., (Mol. Gen. Genet. 248 (1995), 311-317), de Borne et al. (Mol. Gen. Genet. 243 (1994), 613-621) and other sources.

To inhibit the synthesis, in the transformed plants, of a plurality of enzymes involved in starch biosynthesis, it is possible to use DNA molecules for transformation which simultaneously contain, in antisense orientation and under the control of a suitable promoter, a plurality of regions which encode the relevant enzymes. Each sequence may be under the control of its own promoter, or, alternatively, the sequences can be transcribed by a joint promoter as a fusion, so that synthesis of the proteins in question is inhibited to approximately the same or to a different extent. As regards the length of the individual coding regions which are used in such a construct, what has already been said above for the generation of antisense constructs also applies here. In principle, there is no upper limit for the number of antisense fragments transcribed starting from a promoter in such a DNA molecule. However, the resulting transcript should not, as a rule, exceed a length of 25 kb, preferably 15 kb.

The nucleic acid molecules according to the invention make it possible to transform plant cells and simultaneously to inhibit the synthesis of a plurality of enzymes.

Moreover, it is possible to introduce the nucleic acid molecules according to the invention into traditional mutants which are deficient or defective with regard to one or more starch biosynthesis genes (Shannon and Garwood, 1984, in Whistler, BeMiller and Paschall, Starch: Chemistry and Technology, Academic Press, London, 2nd Edition: 25-86). These defects can relate, for example, to the following proteins: granule-bound (GBSS I) and

soluble starch synthases (SSS I, II, III and IV), branching enzymes (BE I, IIa and IIb), debranching enzymes (R-enzymes, isoamylases, pullulanases), disproportioning enzymes and plastid starch phosphorylases.

The present invention thus also relates to transgenic plant cells obtainable by a process according to the invention which have been transformed with a nucleic acid molecule or vector according to the invention, and to transgenic plant cells derived from cells transformed in this way. The cells according to the invention contain a nucleic acid molecule according to the invention, this preferably being linked to regulatory DNA elements which ensure transcription in plant cells, in particular to a promoter. The cells according to the invention can be distinguished from naturally occurring plant cells, inter alia, by the fact that they contain a nucleic acid molecule according to the invention which does not occur naturally in these cells, or by the fact that such a molecule exists integrated at a location in the cell's genome where it does not occur otherwise, i.e. in a different genomic environment. Furthermore, the transgenic plant cells according to the invention can be distinguished from naturally occurring plant cells by the fact that they contain at least one copy of a nucleic acid molecule according to the invention stably integrated into their genome, if appropriate in addition to copies of such a molecule which occur naturally in the cells. If the nucleic acid molecule(s) introduced into the cells is (are) additional copies to molecules which already occur naturally in the cells, then the plant cells according to the invention can be distinguished from naturally occurring plant cells in particular by the fact that this (these) additional copy (copies) is (are) localized at sites of the genome at which it (they) do(es) not occur naturally. This can be checked, for example, with the aid of a Southern blot analysis.

Preferred plant cells according to the invention are those in which the enzyme activity of individual enzymes which are involved in starch metabolism is increased or reduced by at least 10%, especially preferably by at least 30%, and very especially preferably by at least 50%.

Moreover, the plant cells according to the invention can be distinguished from naturally occurring plant cells preferably by at least one of the following features: if the nucleic acid molecule according to the invention

which has been introduced is heterologous relative to the plant cell, the transgenic plant cells exhibit transcripts of the nucleic acid molecules according to the invention which have been introduced. This can be detected by, for example, northern blot analysis. For example, the plant cells according to the invention contain one or more proteins encoded by a nucleic acid molecule according to the invention which has been introduced. This can be detected by, for example, immunological methods, in particular by western blot analysis.

If the nucleic acid molecule according to the invention which has been introduced is homologous relative to the plant cell, the cells according to the invention can be distinguished from naturally occurring cells, for example, on the basis of the additional expression of nucleic acid molecules according to the invention. For example, the transgenic plant cells contain more or fewer transcripts of the nucleic acid molecules according to the invention. This can be detected by, for example, northern blot analysis. "More" or "fewer" in this context means preferably at least 10% more or fewer, preferably at least 20% more or fewer and especially preferably at least 50% more or fewer transcripts than corresponding untransformed cells. Furthermore, the cells preferably exhibit a corresponding (At least 10%, 20% or 50%, respectively) increase or decrease in the content of protein according to the invention. The transgenic plant cells can be regenerated into intact plants by techniques known to the skilled worker.

The plants obtainable by regenerating the transgenic plant cells according to the invention, and processes for the generation of transgenic plants by regenerating intact plants from the plant cells according to the invention, are also subject matter of the present invention. Another subject matter of the invention are plants which contain the transgenic plant cells according to the invention. In principle, the transgenic plants can be plants of any species, i.e. not only monocotyledonous, but also dicotyledonous plants. The plants are preferably useful plants, i.e. plants which are grown by man for the purposes of nutrition or for technical, in particular industrial, purposes. They are preferably starch-storing plants such as, for example, cereal species (rye, barley, oats, maize, wheat, sorghum and millet, sago etc.), rice, peas, marrowfat peas, cassava, potatoes, tomatoes, oilseed

rape, soybeans, hemp, flax, sunflowers, cowpeas, mung beans or arrowroot.

The invention also relates to propagation material of the plants according to the invention, for example fruits, seeds, tubers, root stocks, seedlings, cuttings, calli, protoplasts, cell cultures etc.

Altering the enzymatic activities of the enzymes involved in starch metabolism results in the synthesis, in the plants generated by the process according to the invention, of a starch with a modified structure.

A large number of cloning vectors are available for preparing the introduction of foreign genes into higher plants, vectors which contain a replication signal for *E. coli* and a marker gene for the selection of transformed bacterial cells. Examples of such vectors are pBR322, pUC series, M13mp series, pACYC184 and the like. The desired sequence can be introduced into the vector at a suitable restriction cleavage site. The resulting plasmid is used for transforming *E. coli* cells. Transformed *E. coli* cells are cultured in a suitable medium and then harvested and lysed. The plasmid is recovered. The analytical methods for characterizing the plasmid DNA obtained are generally restriction analyses, gel electrophoreses and other methods of biochemistry and molecular biology (Sambrook et al. loc. cit.). After each manipulation, the plasmid DNA can be cleaved and DNA fragments obtained linked to other DNA sequences. Each plasmid DNA sequence can be cloned into the same or other plasmids.

A large number of techniques are available for introducing DNA into a plant host cell. These techniques encompass the transformation of plant cells with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as the means for transformation, protoplast fusion by means of polyethylene glycol (PEG), injection, DNA electroporation, the introduction of DNA by means of the biolistic method, and other possibilities.

The injection and electroporation of DNA into plant cells requires no particular aspects of the plasmids or the DNA used per se. Simple plasmids such as, for example, pUC derivatives can be used. However, if intact

plants are to be regenerated from such transformed cells, the presence of a selectable marker gene is required.

Depending on the method of introducing desired genes into the plant cell, further DNA sequences may be required. If, for example, the Ti or Ri plasmid is used for transforming the plant cell, at least the right border, but frequently the right and left border, of the Ti and Ri plasmid T-DNA must be linked to the genes to be introduced as flanking region. If agrobacteria are used for the transformation, the DNA to be introduced must be cloned into specific plasmids, either into an intermediate vector or into a binary vector. The intermediate vectors can be integrated into the agrobacterial Ti or Ri plasmid by homologous recombination owing to sequences which are homologous to sequences in the T-DNA. The former also contains the *vir* region required for T-DNA transfer. Intermediate vectors cannot replicate in agrobacteria. The intermediate vector can be transferred to *Agrobacterium tumefaciens* by means of a helper plasmid (conjugation). Binary vectors are capable of replication in *E. coli* and in agrobacteria. They contain a selection marker gene and a linker or polylinker which are framed by the left and right T-DNA border region. They can be transformed directly into the agrobacteria (Holsters et al. (1978) Mol. Gen. Genet. 163: 181-187). The agrobacterium which acts as the host cell should contain a plasmid carrying a *vir* region. The *vir* region is required for transferring the T-DNA into the plant cell. Additional T-DNA may be present. The agrobacterium transformed in this way is used for transforming plant cells.

The use of T-DNA for transforming plant cells has been researched intensively and been described in EP 120516; Hoekema, in: The Binary Plant Vector System Offsetdrukkerij Kanters B.V., Alblasserdam (1985), Chapter V; Fraley et al., Crit. Rev. Plant. Sci., 4: 1-46 and An et al. (1985) EMBO J. 4: 277-287.

To transfer the DNA into the plant cell, plant explants can expediently be cocultured with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*. Intact plants can then be regenerated from the infected plant material (for example leaf sections, stem segments, roots, but also protoplasts, or plant cells which have been grown in suspension culture) in a suitable medium which can contain antibiotics or biocides for selecting transformed cells.

The resulting plants can then be examined for the presence of the DNA which has been introduced. Other possibilities of introducing foreign DNA using the biolistic method or by protoplast transformation are known (cf., for example, Willmitzer, L, 1993 Transgenic plants. In: Biotechnology, A Multi-Volume Comprehensive Treatise (H.J. Rehm, G. Reed, A. Pühler, P. Stadler, eds.), Vol. 2, 627-659, VCH Weinheim-New York-Basle-Cambridge).

While the transformation of dicotyledonous plants via Ti-plasmid vector systems with the aid of Agrobacterium tumefaciens is well established, more recent work suggests that even monocotyledonous plants are indeed accessible to transformation by means of agrobacterium-based vectors (Chan et al., Plant Mol. Biol. 22 (1993), 491-506; Hiei et al., Plant J. 6 (1994), 271-282).

Alternative systems for the transformation of monocotyledonous plants are the transformation by means of the biolistic approach, protoplast transformation, the electroporation of partially permeabilized cells, and the introduction of DNA by means of glass fibers.

Specifically, different methods have been described in the literature for the transformation of maize (cf., for example, WO 95/06128, EP 0 513 849; EP 0 465 875). EP 292 435 describes a method with the aid of which fertile plants can be obtained starting from a mucilage-free, friable, granular maize callus. In this context, Shillito et al. (Bio/Technology 7 (1989), 581) have observed that the capacity of regenerating fertile plants requires starting from callus suspension cultures from which a dividing protoplast culture with the capacity of regenerating plants can be made. Following an in-vitro culture period of 7 to 8 months, Shillito et al. obtained plants with viable progeny which, however, have abnormalities with regard to morphology and reproductivity. Prioli and Söndahl (Bio/Technology 7 (1989), 589) also describe the regeneration and obtaining of fertile maize plants from maize protoplasts.

Once the DNA which has been introduced is integrated into the genome of the plant cell, it is, as a rule, stable therein and is also retained in the progeny of the originally transformed cell. It normally contains a selection

marker which imparts to the transformed plant cells resistance to a biocide or an antibiotic such as kanamycin, G 418, bleomycin, hygromycin or phosphinotricin and the like. The individual marker chosen should therefore allow selection of transformed cells over cells which lack the DNA which has been introduced.

Within the plant, the transformed cells grow in the customary manner (see also McCormick et al. (1986) Plant Cell Reports 5:81-84). The resulting plants can be grown normally and hybridized with plants which have the same transformed germ plasm or other germ plasm. The resulting hybrids have the corresponding phenotypic features.

Two or more generations should be grown to ensure that the phenotypic feature is stably retained and inherited. Also, seeds should be harvested to ensure that the phenotype in question or other features have been retained.

Yet another subject matter of the invention is a process for the production of starch in a manner known per se, in which plant cells according to the invention, plants according to the invention, plant parts according to the invention or propagation material according to the invention are processed or integrated into the process.

Processes for extracting starch from plants or from starch-storing parts of plants are known to the skilled worker. Processes for extracting starch from maize kernels are described, for example, by Eckhoff et al. (Cereal Chem. 73 (1996) 54-57). As a rule, the extraction of maize starch on the industrial scale is performed by wet milling. Moreover, processes for extracting the starch from various starch-storing plants are described, for example, in "Starch: Chemistry and Technology (eds: Whistler, BeMiller and Paschall (1994), 2nd Edition, Academic Press Inc. London Ltd; ISBN 0-12-746270-8; see, for example, Chapter XII, pages 412-468: maize and sorghum starches: production; by Watson; Chapter XIII, pages 469-479: tapioca, arrowroot and sago starches: production; by Corbishley and Miller; Chapter XIV, pages 479-490: potato starch: production and uses; by Mitch; Chapter XV, pages 491 to 506: wheat starch: production, modification and uses; by Knight and Oson; and Chapter XVI, pages 507 to 528: rice starch:

production and uses; by Rohmer and Klem). Devices normally used in processes for extracting starch from plant material are separators, decanters, hydrocyclones, spray dryers and fluidized-bed dryers.

Owing to the expression of a nucleic acid molecule according to the invention, the transgenic plant cells and plants according to the invention synthesize a starch which is modified in comparison to the starch synthesized in wild-type plants for example with regard to its physico-chemical properties.

Yet another subject matter of the invention is the starch which can be obtained from a plant cell according to the invention, plant according to the invention, their propagation material or a method according to the invention.

A further embodiment of the present invention also includes the use of the starch according to the invention in industry for the production of foodstuffs, packaging materials or disposable products.

The starch according to the invention can be modified by processes known to the skilled worker and is suitable, in its unmodified or modified form, for a variety of applications in the food or non-food sector.

In principle, the possible uses of the starch according to the invention can be divided into two important sectors. One sector encompasses the hydrolisates of the starch, mainly glucose and glucose units, which are obtained by enzymatic or chemical methods. They are used as starting material for other chemical modifications and processes such as fermentation. What may be important here is the simplicity and inexpensive design of a hydrolytic process as is currently performed essentially enzymatically using amyloglucosidase. What would be feasible is a financial saving by using less enzyme. This could be caused by altering the structure of the starch, for example increasing the surface area of the granule, by better degradability owing to a lower degree of branching, or by a steric structure which limits the accessibility for the enzymes employed.

The other sector in which starch according to the invention can be used as so-called native starch, due to its polymeric structure, can be divided into two further fields of application:

1. The food industry

Starch is a traditional additive to a large number of foodstuffs in which its function is essentially to bind aqueous additives or to cause increased viscosity or else increased gelling. Important characteristics are the visco-elasticity, the sorptive characteristics, the swelling temperature, the gelatinization temperature, the viscosity and thickening power, starch solubility, transparency and gel structure, thermal stability, shear stability, stability to acids, the tendency to undergo retrogradation, the film-forming capacity, the freeze-thaw stability, digestibility and the ability of forming complexes with, for example, inorganic or organic ions.

2. The non-food industry

In this important sector, starch is employed as an auxiliary for various preparation processes or as an additive in industrial products. When using starch as an auxiliary, mention must be made, in particular, of the paper and board industry. Starch acts mainly for retardation purposes (retaining solids), binding filler particles and fines, as a stiffener and for dehydration. Moreover, the advantageous properties of starch regarding stiffness, strength, sound, touch, luster, smoothness, bonding strength and the surfaces are made use of.

2.1. The paper and board industry

Within the papermaking process, four fields of application must be distinguished, i.e. surface, coating, stock and spraying. With 80% of the consumption, surface starch accounts for by far the greatest starch quantity, 8% are used as coating starch, 7% as stock starch and 5% as spraying starch.

The demands on starch with regard to surface treatment are essentially high whiteness, an adapted viscosity, highly stable viscosity, good film formation and low dust formation. When used for coating, the solids content, an adapted viscosity, a high binding capacity and a high pigment affinity play an important role. Of importance when used as an additive to the stock is rapid, uniform, loss-free distribution, high mechanical strength

and complete retention in the paper web. If the starch is used in the spraying sector, again, an adapted solids content, high viscosity and a high binding capacity are of importance.

2.2. The adhesives industry

An important field of application for starches is in the adhesives industry, where the potential uses can be divided into four subsections: the use as a pure starch paste, the use in starch pastes which have been treated with specialty chemicals, the use of starch as additive to synthetic resins and polymer dispersions, and the use of starches as extenders for synthetic adhesives. 90% of the starch based adhesives is employed in the sectors production of corrugated board, production of paper sacks and bags, production of composite materials for paper and aluminum, production of box board and gumming adhesives for envelopes, stamps and the like.

2.3. The textiles and textile care products industry

An important field of application for starches as auxiliaries and additive is the sector production of textiles and textile care products. The following four fields of application must be distinguished within the textiles industry: the use of starch as sizing agent, i.e. as auxiliary for smoothing and strengthening the burring behavior as a protection from the tensile forces applied during weaving, and for increasing resistance to abrasion during weaving, starch as a textile finishing agent, in particular after quality-reducing pretreatments such as bleaching, dyeing and the like, starch as thickener in the preparation of dye pastes for preventing bleeding, and starch as additive to chaining agents for sewing threads.

2.4. The construction materials industry

The fourth field of application is the use of starches as additive in construction materials. An example is the production of gypsum plasterboards, where the starch which is admixed to the gypsum slurry gelatinizes with the water, diffuses to the surface of the plaster core, where it binds the boards to the core. Other fields of application are the admixture to rendering and mineral fibers. In the case of ready-mixed concrete, starch products are employed for delaying binding.

2.5. Soil stabilization

A limited market for starch products is the production of soil stabilizers, which are employed for the temporary protection of the soil particles from water when the soil is disturbed artificially. According to present knowledge, product combinations of starch and polymer emulsions equal the previously employed products with regard to their erosion- and crust-reducing effect, but are markedly less expensive.

2.6. Use in crop protection products and fertilizers

One field of application for using starch is in crop protection products for altering the specific properties of the products. Thus, starches are employed for improving the wettability of crop protection products and fertilizers, for the controlled release of the active ingredients, for converting liquid active ingredients, volatile active ingredients and/or active ingredients with an offensive odor into microcrystalline, stable, shapeable substances, for mixing incompatible compounds, and for extending the duration of action by reducing decomposition.

2.7. Pharmaceuticals, medicine, and the cosmetics industry

Another field of application is the sector of pharmaceuticals, medicine and the cosmetics industry. In the pharmaceuticals industry, starches are employed as binders for tablets or for diluting the binder in capsules. Moreover, starches are used as tablet disintegrants, since they absorb fluids after swallowing and swell within a short time to such an extent that the active ingredient is liberated. Medicinal lubricating powders and wound powders are starch-based for reasons of quality. In the cosmetics sector, starches are employed, for example, as carriers of powder additives such as fragrances and salicylic acid. A relatively large field of application for starch is toothpaste.

2.8. Addition of starch to coal and briquettes

A field of application for starch is as additive to coal and briquettes. With an addition of starch, coal can be agglomerated, or briquetted, in terms of high quantity, thus preventing early decomposition of the briquettes. In the case of barbecue coal, the starch addition amounts to between 4 and 6%, in the case of calorized coal to between 0.1 and 0.5%. Moreover, starches are gaining importance as binders since the emission of noxious substances can be markedly reduced when starches are added to coal and briquettes.

2.9. Ore slick and coal silt processing

Furthermore, starch can be employed as flocculant in ore slick and coal silt processing.

2.10. Foundry auxiliary

A further field of application is as additive to foundry auxiliaries. Various casting processes require cores made with sands treated with binders. The binder which is predominantly employed nowadays is bentonite, which is treated with modified starches, in most cases swellable starches.

The purpose of adding starch is to increase flowability and to improve the binding power. In addition, the swellable starches can meet the demands of production engineering, such as being cold-water dispersible, rehydratable and readily miscible with sand and having high water binding capacity.

2.11. Use in the rubber industry

In the rubber industry, starch is employed for improving the technical and visual quality. The reasons are the improvement of the surface luster, the improvement of handle and of appearance, and to this end starch is scattered over the tacky gummed surfaces of rubber materials prior to cold curing, and also the improvement of the rubber's printability.

2.12. Production of leather substitutes

Modified starches may furthermore also be sold for the production of leather substitutes.

2.13. Starch in synthetic polymers

In the polymer sector, the following fields of application can be envisaged: the incorporation of starch degradation products in the processing process (starch is only a filler, there is no direct bond between the synthetic polymer and the starch) or, alternatively, the incorporation of starch degradation products in the production of polymers (starch and polymer form a stable bond).

The use of starch as pure filler is not competitive in comparison with other substances such as talc. However, this is different when the specific

properties of starch make an impact and thus markedly alter the spectrum of characteristics of the end products. An example of this is the use of starch products in the processing of thermoplasts, such as polyethylene. Here, the starch and the synthetic polymer are combined by coexpression in the ratio 1:1 to give a master batch, from which various products are produced together with granulated polyethylene, using conventional process techniques. By incorporating starch in polyethylene films, an increased substance permeability in the case of hollow bodies, an improved permeability for water vapor, an improved antistatic behavior, an improved antiblock behavior and an improved printability with aqueous inks can be achieved. The current disadvantages relate to the insufficient transparency, the reduced tensile strength, and a reduced elasticity.

Another possibility is the use of starch in polyurethane foams. By adapting the starch derivatives and by processing-engineering optimization, it is possible to control the reaction between synthetic polymers and the starches' hydroxyl groups in a direct manner. This results in polyurethane films which have the following spectrum of properties, owing to the use of starch: a reduced heat extension coefficient, a reduced shrinking behavior, an improved pressure-tension behavior, an increase in permeability for water vapor without altering the uptake of water, a reduced flammability and a reduced ultimate tensile strength, no drop formation of combustible parts, freedom from halogens, and reduced aging. Disadvantages which still exist are a reduced printability and a reduced impact strength.
Product development is currently no longer restricted to films. Solid polymer products such as pots, slabs and dishes with a starch content of over 50% may also be produced. Moreover, starch/polymer mixtures are considered advantageous since their biodegradability is much higher.

Starch graft polymers have become exceedingly important owing to their extremely high water binding capacity. They are products with a starch backbone and a side chain of a synthetic monomer, grafted on using the principle of the free-radical chain mechanism. The starch graft polymers which are currently available are distinguished by a better binding and retention capacity of up to 1000 g water per g of starch, combined with high viscosity. The fields of application for these superabsorbers have extended

greatly in recent years and are, in the hygiene sector, the products diapers and pads, and, in the agricultural sector, for example in seed coatings.

What is decisive for the application of novel, genetically modified starches are, on the one hand, structure, water content, protein content, lipid content, fiber content, ash/phosphate content, amylose/amylopectin ratio, molecular mass distribution, the degree of branching, granule size, granule shape and crystallization, and, on the other hand, also the characteristics which affect the following features: viscoelasticity, sorption characteristics, gelatinization temperature, viscosity, thickening powder, solubility, gel structure, transparency, thermal stability, shear stability, stability to acids, tendency to undergo retrogradation, gel formation, freeze-thaw stability, complex formation, iodine binding, film formation, adhesive power, enzyme stability, digestibility and reactivity.

The production of modified starches by recombinant methods can, on the one hand, alter the properties, for example of the starch derived from the plant, in such a way that other modifications by means of chemical or physical alterations are no longer required. On the other hand, starches which have been modified by recombinant methods may be subjected to further chemical modifications, which leads to further improvements in quality for some of the above-described fields of application. These chemical modifications are known in principle. They are, in particular, modifications by thermal and pressure treatment, treatment with organic or inorganic acids, enzymatic treatment, oxidations or esterifications, which lead, for example, to the formation of phosphate starches, nitrate starches, sulfate starches, xanthate starches, acetate starches and citrate starches. Moreover, mono- or polyhydric alcohols in the presence of strong acids may be employed for producing starch ethers, resulting in starch alkyl-ethers, O-allyl ethers, hydroxyalkyl ethers, O-carboxylmethyl ethers, N-containing starch ethers, P-containing starch ethers, S-containing starch ethers, crosslinked starches or starch graft polymers.

A use of the starches according to the invention is in industrial application, preferably for foodstuffs or the production of packaging materials and dispersible articles.

The examples which follow serve to illustrate the invention and constitute in no way a restriction.

Abbreviations used:

BE	branching enzyme
bp	base pair
IPTG	isopropyl β-D-thiogalactopyranoside
SS	soluble starch synthase
PMSF	phenylmethylsulfonyl fluoride

Media and solutions used in the examples:

20 x SSC	175.3 g NaCl 88.2 g sodium citrate to 1000 ml with twice-distilled H_2O pH 7.0 with 10 N NaOH
Buffer A	50 mM Tris-HCl pH 8.0 2.5 mM DTT 2 mM EDTA 0.4 mM PMSF 10% glycerol 0.1% sodium dithionite
Buffer B	50 mM Tris-HCl pH 7.6 2.5 mM DTT 2 mM EDTA
Buffer C	0.5 M sodium citrate pH 7.6 50 mM Tris-HCl pH 7.6 2.5 mM DTT 2 mM EDTA
10 x TBS	0.2 M Tris-HCl pH 7.5 5.0 M NaCl
10 x TBST	10 x TBS 0.1% (v/v) Tween 20

Elution buffer 25 mM Tris pH 8.3
250 mM glycine

Dialysis buffer 50 mM Tris-HCl pH 7.0
50 mM NaCl
2 mM EDTA
14.7 mM β-mercaptoethanol
0.5 mM PMSF

Protein buffer 50 mM sodium phosphate buffer pH 7.2
10 mM EDTA
0.5 mM PMSF
14.7 mM β-mercaptoethanol

Description of the figures:

Fig. 1 represents a schematic RVA temperature profile (viscosity vs. time [min]) with the viscosimetric parameters T = gelatinization temperature, temperature at the point in time when gelatinization starts; Max specifies the maximum viscosity; Min specifies the minimum viscosity; Fin specifies the viscosity at the end of the measurement; Set is the difference (Δ) of Min and Fin (setback).

The following methods were used in the examples:

1. Cloning method
The vector pBluescript II SK (Stratagene) was used for cloning into *E. coli*.

For the transformation of plants, the gene constructions were cloned into the binary vector pBinAR Hyg (Höfgen & Willmitzer, 1990, Plant Sci. 66:221-230) and pBinB33-Hyg.

2. Bacterial strains and plasmids
The *E. coli* strain DH5α (Bethesda Research Laboratories, Gaithersburgh, USA) was used for the Bluescript vector p Bluescript II KS (Stratagene)

and for the pBinAR Hyg and pBinB33 Hyg constructs. The *E. coli* strain XL1-Blue was used for the *in vivo* exclusion.

pBinAR

The plasmid pBinAR is a derivative of the binary vector plasmid pBin19 (Bevan, 1984, Nucl. Acid Res. 12:8711-8721), which was constructed as follows: a 529 bp fragment encompassing the nucleotides 6909-7437 of the cauliflower mosaic virus promoter 35S promoter was isolated from plasmid pDH51 as EcoRI/KpnI fragment (Pietrzak et al., 1986), ligated between the EcoRI and KpnI cleavage sites of the pUC18 polylinker, and was termed plasmid pUC18-35S. With the aid of the restriction endonucleases HindIII and PvuII, a 192 bp fragment was isolated from plasmid pAGV40 (Herrera-Estrella et al., 1983), which encompasses DNA of the Ti-plasmid pTiACH5 (Gielen et al, 1984, EMBO J.:835-846) (nucleotides 11749-11939). After the PvuII cleavage sites had been provided with SphI linkers, the fragment was ligated between the SpHI and HindIII cleavage sites of pUC18-35S, and this was termed plasmid pA7. Moreover, the entire polylinker comprising the 35S promoter and the ocs terminator was excised with EcoRI and HindIII and ligated into the appropriately cleaved pBin19. This gave rise to the plant expression vector pBinAR (Höfgen and Willmitzer, 1990).

pBinB33

The promoter of the Solanum tuberosum patatin gene B33 (Rocha-Sosa et al., 1989) was ligated, as DraI fragment (nucleotides -1512 - +14) into the vector pUC19, which had been cleaved with Sst I and which had been made blunt-ended with the aid of T4-DNA polymerase. This gave rise to plasmid pUC19-B33. The B33 promoter was excised from this plasmid with EcoRI and SmaI and ligated into the appropriately cleaved vector pBinAR. This gave rise to the plant expression vector pBinB33.

pBinAR-Hyg

Starting from plasmid pA7 (cf. description of vector pBinAR), the EcoRI-HindIII fragment comprising the 35S promoter, the ocs terminator and the portion of the polylinker situated between the 35S promoter and the ocs terminator was introduced into the appropriately cleaved plasmid pBin-Hyg.

pBinB33-Hyg
Starting from plasmid pBinB33, the EcoRI-HindIII fragment comprising the B33 promoter, part of the polylinker and the ocs terminator was excised and ligated into the appropriate cleaved vector pBin-Hyg. This gave rise to the plant expression vector pBinB33-Hyg.

3. Transformation of *Agrobacterium tumefaciens*
The DNA was transferred by direct transformation using the method of Höfgen&Willmitzer (1988, Nucleic Acids Res. 16:9877). The plasmid DNA of transformed agrobacteria was isolated using the method of Birnboim&Doly (1979, Nucleic Acids Res. 7:1513-1523), subjected to suitable restriction cleavage, and then analyzed by gel electrophoresis.

4. Transformation of potatoes
The transformation of the plasmids into the potato plants (Solanum tuberosum L.cv. Desiree, Vereinigte Saatzuchten eG, Ebstorf) was carried out with the aid of the *Agrobacterium tumefaciens* strain C58C1 (Dietze et al. (1995) in Gene Transfer to Plants. pp. 24-29, eds.: Potrykus, I. and Spangenberg, G., Springer Verlag, Deblaere et al., 1985, Nucl. Acids Res. 13:4777-4788).

Ten small leaves of a sterile potato culture which had been scarified with a scalpel were placed into 10 ml of MS medium (Murashige&Skoog (1962) Physiol. Plant. 15: 473) supplemented with 2% sucrose and containing 50 ml of an *Agrobacterium tumefaciens* overnight culture grown under selection conditions. After the culture had been shaken gently for 3-5 minutes, it was incubated for 2 more days in the dark. For callus induction, the leaves were then placed on MS medium supplemented with 1.6% glucose, 5 mg/l naphthylacetic acid, 0.2 mg/l benzylaminopurin, 250 mg/l claforan, 50 mg/l kanamycin, and 0.80% Bacto agar. After the leaves had been incubated for one week at 25°C and 3000 Lux, they were placed on MS medium supplemented with 1.6% glucose, 1.4 mg/l zeatin ribose, 20 mg/l naphthylacetic acid, 20 mg/l giberellic acid, 250 mg/l claforan, 50 mg/l kanamycin and 0.80% Bacto agar, to induce shoots.

5. Plant culture regime
Potato plants were kept in the greenhouse under the following conditions:

light period	16 h at 25,000 Lux and 22°C
dark period	8 h at 15°C
atmospheric humidity	60%

6. Radiolabeling of DNA fragments

The DNA fragments were radiolabeled with the aid of a DNA Random Primer Labeling Kit by Boehringer Mannheim (Germany) following the manufacturer's instructions.

7. Determination of starch synthase activity

Determination of starch synthase activity was done by determining the incorporation of ^{14}C glucose from ADP[^{14}C glucose] into a product which is insoluble in methanol/KCl, as described by Denyer & Smith, 1992, Planta 186:609-617.

8. Detection of soluble starch synthases in the native gel

To detect the activity of soluble starch synthases by non-denaturing gel electrophoresis, tissue samples of potato tubers were hydrolyzed in 50 mM Tris-HCl pH 7.6, 2 mM DTT, 2.5 mM EDTA, 10% glycerol and 0.4 mM PMSF. The electrophoresis was carried out in a MiniProtean II chamber (BioRAD). The monomer concentration of the gels, which had a thickness of 1.5 mm, was 7.5% (w/v), and 25 mM Tris-glycine pH 8.4 was used as gel buffer and running buffer. Identical amounts of protein extract were applied and separated for 2 hours at 10 mA per gel.

The activity gels were subsequently incubated in 50 mM Tricine-NaOH pH 8.5, 25 mM potassium acetate, 2 mM EDTA, 2 mM DTT, 1 mM ADP-glucose, 0.1% (w/v) amylopectin and 0.5 M sodium citrate. The glucans formed were stained with Lugol's solution.

9. Starch analysis

The starch formed by the transgenic potato plants was characterized by the following methods:

a) Determination of the amylose/amylopectin ratio in starch from potato plants

Starch was isolated from potato plants by standard methods, and the amylose:amylopectin ratio was determined by the method described by Hovenkamp-Hermelink et al. (Potato Research 31 (1988) 241-246).

b) Determination of the phosphate content

In potato starch, some glucose units may be phosphorylated on the carbon atoms of position C2, C3 and C6. To determine the degree of phosphorylation at position C6 of the glucose, 100 mg of starch were hydrolyzed for 4 hours at 95°C in 1 ml of 0.7 M HCl (Nielsen et al. (1994) Plant Physiol. 105: 111-117). Following neutralization with 0.7 M KOH, 50 ml of the hydrolysate were subjected to a visual-enzymatic test to determine glucose-6-phosphate. The change in absorption of the test batch (100 mM imidazole/HCl; 10 mM $MgCl_2$; 0.4 mM NAD; 2 units *Leuconostoc mesteroides* glucose-6-phosphate dehydrogenase; 30°C) was monitored at 334 nm.

The total phosphate was determined as described by Ames, 1996, Methods in Enzymology VIII, 115-118.

c) Analysis of the amylopectin side chains

To analyze distribution and length of the side chains in the starch samples, 1 ml of a 0.1% starch solution was digested with 0.4 U of isoamylase (Megazyme International Ireland Ltd., Bray, Ireland) overnight at 37°C in 100 mM sodium citrate buffer, pH 3.5.

The rest of the analysis was carried out as described by von Tomlinson et al., (1997), Plant J. 11:31-47, unless otherwise specified.

d) Granule size determination

The granule size was determined using a "Lumosed" photosedimentometer by Retsch GmbH, Germany. To this end, 0.2 g of starch was suspended in approx. 150 ml of water and immediately measured. The program supplied by the manufacturer calculated the mean diameter of the starch granules, assuming an average starch density of 1.5 g/l.

e) Gelatinization properties

The gelatinization or viscosity properties of the starch were recorded using a Viscograph E by Brabender oHG, Germany, or a Rapid Visco Analyser, Newport Scientific Pty Ltd., Investment Support Group, Warriewood NSW 2102, Australia. When using the Viscograph E, a suspension of 30 g of starch in 450 ml of water was subjected to the following heating program: heat from 50°C to 96°C at 3°/min, hold for 30 minutes, cool to 30°C at 3°/min and hold again for 30 minutes. The temperature profile gave characteristic gelatinization properties.

When measuring using the Rapid Visco Analysers (RVA) a suspension of 2 g of starch in 25 ml of water was subjected to the following heating program: suspend for 60 seconds at 50°C, heat from 50°C to 95°C at 12°/min, hold for 2.5 minutes, cool to 50°C at 12°C/min and hold again for 2 minutes. The RVA temperature profile gave the viscosimetric parameters of the tested starches for the maximum viscosity (Max), the end viscosity (Fin), the gelatinization temperature (T), the minimum viscosity (Min) occurring after the maximum viscosity and the difference between minimum viscosity and end viscosity (Setback, Set) (cf. Table 1 and Fig. 1).

f) Determination of the gel strength

To determine the gel strength by means of a Texture Analyser, 2 g of starch were gelatinized in 25 ml of water (cf. RVA measurement) and then stored for 24 hours in a sealed container at 25°C with the exclusion of air. The samples were mounted underneath the probe (circular stamp) of a TA-XT2 Texture Analyser (Stable Micro Systems), and the gel strength was determined with the following parameter settings:

Test speed	0.5 mm
Penetration depth	7 mm
Contact area (of the stamp)	113 mm^2
Pressure/contact area	2 g

10. Determination of glucose, fructose and sucrose

To determine the glucose, fructose and sucrose content, small tuber portions (diameter approx. 10 mm) of potato tubers were frozen in liquid nitrogen and then extracted for 30 minutes at 80°C in 0.5 ml of 10 mM HEPES, pH 7.5; 80% (vol/vol) ethanol. The supernatant, which contains

the solubles, was removed and the volume was determined. The supernatant was used for determining the amount of soluble sugars. The quantitative determination of soluble glucose, fructose and sucrose was carried out in a batch of the following composition

100.0	mM imidazole/HCl, pH 6.9
1.5	mM $MgCl_2$
0.5	mM $NADP^+$
1.3	mM ATP
10-50	µl sample
1.0	U yeast glucose-6-phosphate dehydrogenase

The batch was incubated for 5 minutes at room temperature. The sugars were subsequently determined photometrically by measuring the absorption at 340 nm after the successive addition of
1.0 units yeast hexokinase (to determine glucose),
1.0 units yeast phosphoglucoisomerase (to determine fructose), and
1.0 units yeast invertase (to determine sucrose).

Use Examples:

Example 1: Isolation of a cDNA fragment encoding potato α-glucosidase

Total RNA of potato tuber tissue directly underneath (approx. 1 cm) germinating shoots were prepared by standard methods (Sambrook et al., 1989).
The purified total RNA was used as starting material for the preparation of poly A+ RNA (Oligotex, mRNA Purification Kit, in accordance with the manufacturer's instructions). 5 µg of this poly A+ RNA were used to generate a cDNA library (λ ZAPII, Stratagene).
Approximately 3 x 105 plaque-forming units (pfus) of this unamplified cDNA library (primary library) were plated following the manufacturer's instructions (Stratagene) for plaque lifting. The radiolabeled probe (Random Primed DNA Labeling Kit, following the manufacturer's instructions) used for plaque hybridization was the sequence of Genbank Accession No. T76451. The filters were prehybridized for 4 hours at 42°C (buffer: 5 x SSC, 0.5% BSA, 5 x Denhardt, 1% SDS, 40 mM phosphate

buffer, pH 7.2, 100 mg/l herring sperm DNA, 25% formamide) and subsequently hybridized for 14 hours at the same temperature. After hybridization, the filters were washed 3x for 20 minutes with 3x SSC, 0.5% SDS at 42°C and autoradiographed. Hybridizing plaques were singled out, and the phages isolated were used for in-vivo excision following the manufacturer's instructions. Plasmid DNA from the bacterial colonies obtained were isolated, employed for sequence analysis and identified as Seq ID No. 1.

A plasmid DNA isolated in this manner was deposited on 07.24.98 at the Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSMZ) Brunswick, FRG, under the number DSM 12347.

Example 2: Preparation of plasmid p35SαSSI-Hyg

A 1831 bp Asp718/XbaI fragment containing a partial cDNA encoding the potato SSS I (Abel, G., (1995) PhD Thesis, Free University of Berlin), was inserted between the Asp 718 and XbaI cleavage site of the vector pBinAR-Hyg in antisense orientation relative to the 35S promoter.

Example 3: Preparation of plasmid p35S-SSI-Kan

A 2384 bp EcoRI fragment containing a cDNA encoding potato SSI (Abel 1995, loc. cit.) was made blunt-ended and introduced into the vector pBinAR, which had previously been cut with SmaI, in sense orientation relative to the 35S promoter.

Example 4: Preparation of plasmid p35SαSSII-Kan

A 1959 bp SmaI/Asp718 fragment containing a partial cDNA encoding the potato SS II (Abel, 1995, termed GBSS II therein) was made blunt-ended and introduced into the SmaI cleavage site of the vector pBinAR in anti-sense orientation relative to the 35S promoter.

Example 5: Preparation of plasmid pB33-SSII-Hyg

A 2619 bp SmaI/SalI fragment containing a cDNA encoding the potato SS II (Abel, 1995, loc. cit.) was introduced into the vector pBinB33-Hyg, which had previously been cut with SmaI and SalI in sense orientation relative to the B33 promoter.

Example 6: Preparation of plasmid p35SαSSIII-Hyg

A 4212 bp Asp718/XbaI fragment containing a cDNA encoding the potato SS III (Abel et al., 1996, Plant J. 10(6):981-991), was inserted between the Asp718 and the XbaI cleavage site of the vector pBinAR-Hyg in antisense orientation relative to the 35S promoter.

Example 7: Preparation of plasmid p35S-SSIII-Kan

A 4191 bp EcoRI fragment containing a cDNA encoding potato SS III (Abel et al., 1996, loc. cit.), was made blunt-ended and introduced into the SmaI cleavage site of the vector pBinAR in sense orientation relative to the 35S promoter.

Example 8: Preparation of plasmid pB33αBEαSSIII-Kan

A 1650 bp HindII fragment which contains a partial cDNA encoding the potato BE enzyme (Kossmann et al., 1991, Mol. & Gen. Genetics 230(1-2):39-44) was made blunt-ended and introduced in antisense orientation relative to the B33 promoter into the vector pBinB33 which had been precut with SmaI. The resulting plasmid was cut open with BamHI. A 1362 Bp BamHI fragment containing a partial cDNA encoding the potato SS III enzyme (Abel et al., 1996, loc. cit.) was introduced into the cleavage site, again in antisense orientation relative to the B33 promoter.

Example 9: Preparation of plasmid p35SαSSII-αSSIII-Kan

A 1546 bp EcoRV/HincII fragment containing a partial cDNA encoding the potato SS II (Abel, 1995, loc. cit.) was cloned into the vector pBluescript II KS which can been cut with EcoRV/HincII, then excised again by digestion with Asp718/BamHI and introduced in antisense orientation relative to the 35S promoter into the vector pBinAR which had been digested in the same

manner. Then, a 1356 bp BamHI fragment containing a partial cDNA encoding the potato SS III (Abel et al., 1996, loc. cit.), was introduced into the BamHI cleavage site of the vector pBinAR-αSSII, again in antisense orientation.

Example 10: Preparation of plasmid pB33αSSIαSSIαSSIII-Kan

A 2384 bp EcoRI fragment containing a cDNA encoding the potato SS I (Abel, 1995, loc. cit.) was made blunt-ended and cloned into the SmaI cleavage site of the pBinB33 vector in antisense orientation relative to the B33 promoter. A 1362 bp BamHI fragment containing a partial cDNA encoding the potato SS III (Abel et al., 1996, loc. cit.) was introduced into the BamHI cleavage site of the resulting vector, again in antisense orientation relative to the B33 promoter.

Example 11: Preparation of plasmid p35SαSSII-Hyg

A 1959 bp SmaI/Asp718 fragment containing a partial cDNA encoding the SS II (Abel, 1995, loc. cit.), was made blunt-ended and introduced into the SmaI cleavage site of the pBinAR-Hyg vector in antisense orientation relative to the 35S promoter.

Example 12: Introduction of the plasmids into the genome of potato cells

The plasmids stated in Examples 1 to 11 were transferred, either individually and/or in succession, into agrobacteria, with the aid of which potato cells were transformed as described above. Subsequently, intact plants were regenerated from the transformed plant cells.

Example 13: Characterization of the physico-chemical properties of the modified starches

As a result of the transformation, the transgenic potato plants showed a change in the physico-chemical properties of the starches synthesized by them. The starch formed by these plants differs for example from starch synthesized in wild-type plants with regard to its phosphate or amylose

content, the viscosity or gelatinization properties determined by RVA, and its chromatographic behavior.

[changes to the form on p 61/2]

Name and address: copy "Hoechst ... am Main"

Under I:
Reference: "St-Glu18"
Number: "DSM 12347"

Under II:
Put an "x" against "A proposed taxonomic designation"

Under III:
Date: "1998-07-24"

Under V:
Name: copy "DSMZ ... GmbH"
Address: copy "Mascheroder ... Brunswick"
Date: "1998-07-30"
Name: "[signature]"

We claim:

1. A nucleic acid molecule encoding a protein with the function of a potato α-glucosidase, selected from the group consisting of
a) nucleic acid molecules which encode a protein which encompasses the amino acid sequence stated under Seq ID NO. 2 or its derivatives or parts,
b) nucleic acid molecules which encompass the nucleotide sequence shown under Seq ID No. 1 or its derivatives or parts, or a corresponding ribonucleotide sequence;
c) nucleic acid molecules which hybridize with, preferably which hybridize specifically with, or are complementary to, the nucleic acid molecules stated under a) or b), and
d) nucleic acid molecules whose nucleotide sequence deviates from the sequence of the nucleic acid molecules stated under a), b) or c) owing to the degeneracy of the genetic code.

2. A recombinant nucleic acid molecule containing
a) a nucleic acid molecule encoding a protein with the function of a potato α-glucosidase as claimed in claim 1 and
b) one or more nucleotide sequences which encode a protein selected from amongst group A, composed of proteins with the function of branching enzymes, ADP glucose pyrophoshorylases, granule-bound starch synthases, soluble starch synthases, debranching enzymes, disproportioning enzymes, plastid starch phosphorylases, R1-enzymes, amylases, glucosidases, parts of said nucleotide sequences, or nucleic acid molecules which hybridize with said nucleotide sequences.

3. A nucleic acid molecule as claimed in claim 1 or 2, which is a deoxyribonucleic acid molecule.

4. A nucleic acid molecule as claimed in claim 2, which is a cDNA molecule.

5. A nucleic acid molecule as claimed in claim 1, which is a ribonucleic acid molecule.

6. A nucleic acid molecule which hybridizes, preferably specifically hybridizes, with a nucleic acid molecule [lacuna] one or more of claims 1 to 5.

7. A vector comprising a nucleic acid molecule as claimed in one or more of claims 1 to 6.

8. A vector comprising a nucleic acid molecule as claimed in one or more of claims 1-6, wherein the nucleotide sequence encoding a protein with the function of a soluble starch synthase III or parts thereof is present in sense or antisense orientation.

9. A vector comprising a nucleic acid molecule as claimed in one or more of claims 1-6, wherein the nucleotide sequence encoding one or more proteins selected from group A or parts thereof is present in sense or antisense orientation.

10. A vector comprising a nucleic acid molecule as claimed in one or more of claims 1-6, wherein the nucleotide sequence encoding one or more proteins selected from group A is partly present in sense orientation and partly in antisense orientation.

11. A vector comprising a nucleic acid molecule as claimed in one or more of claims 1-6, which is linked to regulatory elements which ensure transcription and synthesis of an RNA, which is optionally translatable, in a pro- or eukaryotic cell.

12. A host cell which is transformed with a nucleic acid molecule as claimed in one or more of claims 1-6 or a vector as claimed in one or more of claims 7-11 or which is derived from such a cell.

13. A process for the generation of a transgenic plant cell which synthesizes a modified starch, wherein a nucleic acid molecule as claimed in one or more of claims 1-6 or a vector as claimed in claim 7-11 is integrated into the genome of a plant cell.

14. A plant cell which is obtainable by a process as claimed in claim 13.

15. A process for generating a transgenic plant which synthesizes a modified starch, wherein an intact plant is regenerated from a cell as claimed in claim 14.

16. A plant comprising a plant cell as claimed in claim 14.

17. A plant as claimed in claim 16, which is a useful plant.

18. A plant as claimed in one or more of claims 16 to 17, which is a starch-storing plant.

19. A plant as claimed in one or more of claims 16 to 18, which is a wheat, maize, potato or rice plant.

20. Propagation material of a plant as claimed in one or more of claims 16 to 19.

21. A process for the production of starch by a method known per se, wherein plant cells as claimed in claim 14, plants as claimed in one or more of claims 16 to 19 or propagation material as claimed in claim 20 are integrated into the process.

22. A starch obtainable from a cell as claimed in claim 12 or 14, a plant as claimed in one or more of claims 16 to 19, from propagation material as claimed in claim 20 or a process as claimed in claim 21.

23. The use of the starch as claimed in claim 22 in the industrial sector, preferably for the production of foodstuffs, packaging materials or disposable articles.

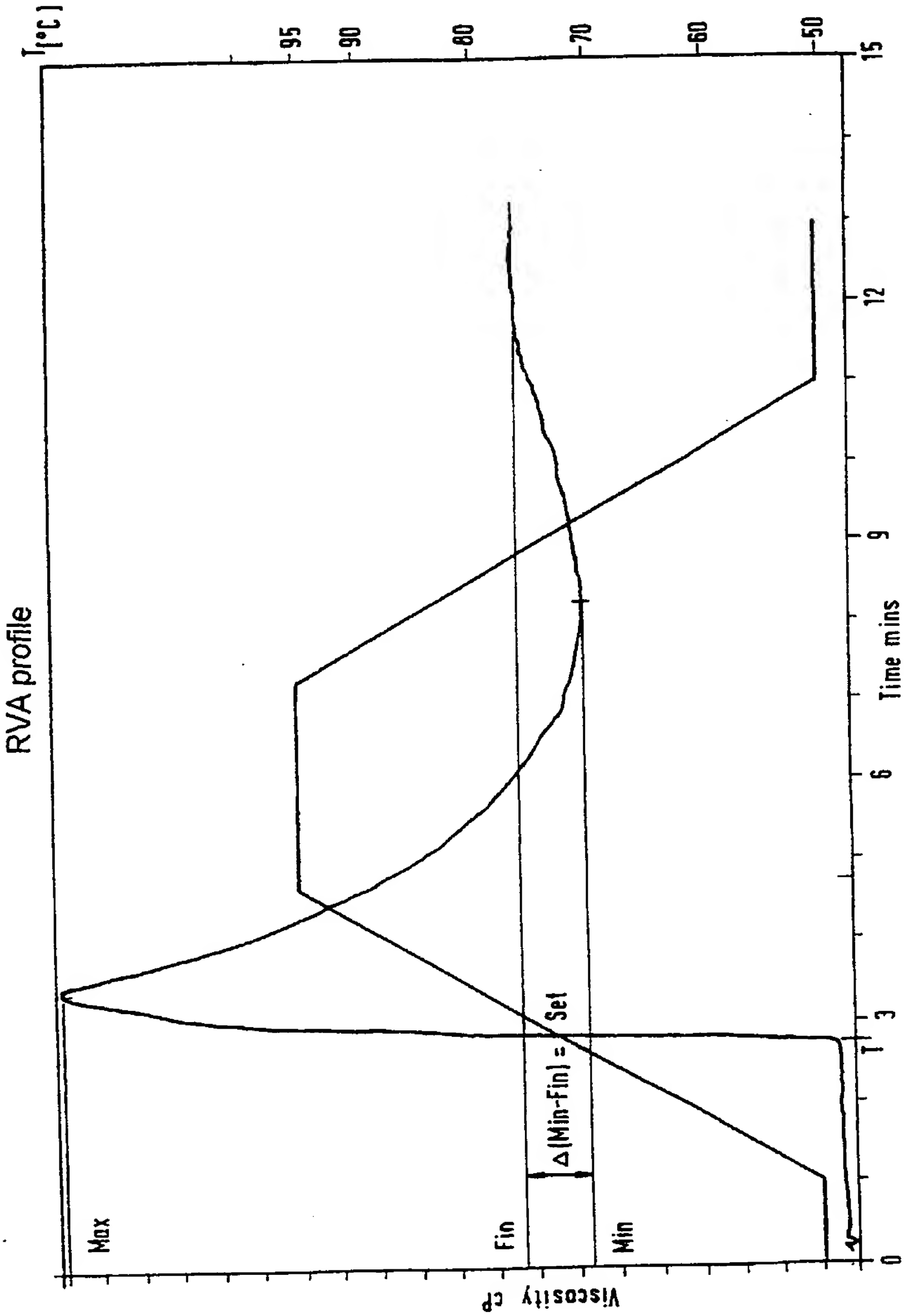
24. The use of nucleic acid molecules as claimed in one or more of claims 1-6 or vectors as claimed in one or more of claims 7-11 for the generation of transgenic cells, preferably bacterial or plant cells.

25. The use of plant cells as claimed in claim 14, plants as claimed in one or more of claims 16 to 19 or propagation material as claimed in claim 20 for the production of starch.

Abstract

Nucl ic acid molecules encoding an α-glucosidase, plants which synthesize a modified starch, the generation of the plants, their use, and the modified starch

The present invention relates to nucleic acid molecules which encode a protein with the activity of a potato α-glucosidase and to processes for the generation of transgenic plant cells and plants which synthesize a modified starch. Moreover, the present invention relates to vectors and host cells comprising the nucleic acid molecules according to the invention, to the plant cells and plants originating from the processes according to the invention, to the starch synthesized by the plant cells and plants according to the invention, and to processes for the production of this starch.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International Bureau

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Berlin(DE).

(81) Designated states: AE, AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GD, GE, HR, HU, ID, IL, IN, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, TJ, TM, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO Patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian Patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European Patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, LU, MC, NL, PT, SE), OAPI Patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
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As printed

(54) Title: NUCLEIC ACID MODULE CODING FOR ALPHA GLUCOSIDASE, PLANTS THAT SYNTHESIZE MODIFIED STARCH, METHODS FOR THE PRODUCTION AND USE OF SAID PLANTS, AND MODIFIED STARCH

(54) Bezeichnung: NUKLEINSÄUREMOLEKÜLE KODIEREND FÜR EINE α–GLUKOSIDASE, PFLANZEN, DIE EINE MODIFIZIERTE STÄRKE SYNTHETISIEREN, VERFAHREN ZUR HERSTELLUNG DER PFLANZEN, IHRE VERWENDUNG SOWIE DIE MODIFIZIERTE STÄRKE

(57) Abstract

The present invention relates to nucleic acid molecules coding for a protein with the activity of an alpha–glucosidase from a potato.The invention further relates to methods for the production of transgenic plant cells and plants synthesizing modified starch. The invention further relates to vectors and host cells containing the inventive nucleic acid modules, plant cells and plants obtained according to the inventive methods, starch synthesized by the inventive plant cells and methods for the production of said starch.

(57) Zusammenfassung

Die vorliegende Erfindung betrifft Nukleinsäuremoleküle, die ein Protein mit der Aktivität einer α–Glukosidase aus Kartoffel kodieren sowie Verfahren zur Herstellung transgener Pflanzenzellen und Pflanzen, die eine modifizierte Stärke synthetisieren. Des weiteren betrifft die vorliegende Erfindung Vektoren und Wirtszellen, welche die erfindungsgemäßen Nukleinsäuremoleküle enthalten, die aus den erfindungsgemäßen Verfahren hervorgehenden Pflanzenzellen und Pflanzen, die von den erfindungsgemäßen Pflanzenzellen und Pflanzen synthetisierte Stärke sowie Verfahren zur Herstellung dieser Stärke.

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 1998/M225 PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP99/05536	International filing date (*day/month/year*) 30 July 1999 (30.07.99)	Priority date (*day/month/year*) 31 July 1998 (31.07.98)
International Patent Classification (IPC) or national classification and IPC C12N 15/55		
Applicant AVENTIS CROPSCIENCE GMBH		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

☒ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 29 January 2000 (29.01.00)	Date of completion of this report 23 November 2000 (23.11.2000)
Name and mailing address of the IPEA/EP Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/EP99/05536

I. Basis of the report

1. With regard to the **elements** of the international application:*

☐ the international application as originally filed

☒ the description:

pages 1-58, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the claims:

pages 12-25, as originally filed
pages _____, as amended (together with any statement under Article 19
pages _____, filed with the demand
pages 1-11, filed with the letter of 13 November 2000 (13.11.2000)

☒ the drawings:

pages 1/1, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

☒ the sequence listing part of the description:

pages 1-5, as originally filed
pages _____, filed with the demand
pages _____, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____
☐ the claims, Nos. _____
☐ the drawings, sheets/fig _____

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rule 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

[redacted]national application No.
PCT/EP 99/05536

I. Basis of the report

1. This report has been drawn on the basis of *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

The sequence protocol on pages 1-5 was part of the application and was taken into consideration during the examination.

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 2-4,6,7,9-25

because:

☐ the said international application, or the said claims Nos. _____
relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 22,23
are so unclear that no meaningful opinion could be formed *(specify)*:

See annex

☐ the claims, or said claims Nos. _____ are so inadequately supported
by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. 2-4,6,7,9-25 .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of:BOX III

1. The examination of Claims 2-4, 6, 7 and 9-25 was carried out only within the limits covered by the international search report (PCT Rule 66.1(e)). In the present case, the examination of the above-mentioned claims concerned the α-glucosidase enzyme and the combination of α-glucosidase with various soluble starch synthases (SS I, SS II, SS III) and with the branching enzyme (BE). As explained in the search report, the search was based on those nucleic acid molecules, methods and plants which can be regarded as being supported by the description (PCT Article 6) and as sufficiently disclosed (PCT Article 5). In the present case, these are the nucleic acids, methods and plants presented in the Examples 1-12.

   The examination of non-searched subject matter is not possible.

2. Claims 22 and 23 are unclear (see Box VIII) and do not contain any technical features. In Boxes V and VIII, the content of the claims is discussed as much as possible. However, owing to the lack of technical features, a conclusive examination is not possible.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

ernational application No.
PCT/EP 99/05536

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	2, 8-10, 21	**YES**
	Claims	1, 3-7, 11-20, 22-25	**NO**
Inventive step (IS)	Claims		**YES**
	Claims	1-25	**NO**
Industrial applicability (IA)	Claims	1-25	**YES**
	Claims		**NO**

2. Citations and explanations

This report makes reference to the following documents:

D1: SUGIMOTO M. ET AL.: "Molecular cloning and characterization of a cDNA encoding alpha-glucosidase from spinach", PLANT MOLECULAR BIOLOGY, Vol. 33, 1997, pages 765-768, mentioned in the application

D2: US-A-5 763 252 (TIBBOT BRIAN K. ET AL.), 9 June 1998 (1998-06-09)

D3: WO-A-97/24448 (NICKERSON BIOCEM LTD; TAYLOR MARK ANDREW (GB); DAVIES HOWARD VIVIA), 10 July 1997 (1997-07-10)

D4: WO-A-94/09144 (ZENECA LTD), 28 April 1994 (1994-04-28)

D5: WO-A-95/07355 (INST. GENBIOLOGISCHE FORSCHUNG; KOSSMANN JENS (DE); VIRGIN IVAR (DE)); 16 March 1995 (1995-03-16), mentioned in the application

D6: WO-A-97/11188 (KOSSMANN JENS; LORBERTH RUTH (DE); PLANTTEC BIOTECHNOLOGIE GMBH (D)), 27 March 1997 (1997-03-27), mentioned in the application

D7: WO-A-96/15248 (ABEL GERNOT J.; INST. GENBIOLOGISCHE FORSCHUNG (DE); KOSSMANN JENS (DE)); 23 May 1996 (1996-05-23), mentioned in the application.

The present application concerns an α-glucosidase, the nucleic acid molecules that code for this α-glucosidase, and combinations of this α-glucosidase with other enzymes involved in starch formation.

1. The amendments submitted by fax on 13 November 2000 comply with PCT Articles 19(2) and 34(2)(b) and are therefore admissible.

2. Novelty (PCT Article 33(2)):

2.1 D1, D2 and D3 each disclose an α-glucosidase isolated from a different source. The α-glucosidase in D1 originates from spinach and shows 62% sequence identity at the amino acid level and 68% sequence identity at the nucleic acid level. The enzyme in D2 was isolated from barley and shows approximately 58% sequence identity at both the amino acid and nucleotide levels. The α-glucosidase disclosed in D3 originates from potatoes and shows 33% identity at the amino acid level with the enzyme of the present application. The sequences disclosed in D1-D3 are not identical to the seq. ID No. 1, but can be regarded as derivatives having the function of an α-glucosidase, this view being further encouraged by unclear formulations (see Box VIII). All the three said enzymes and their coding sequences therefore meet the requirement of Claim 1(a) and 1(d).

It is noted that the present formulation of Claim 1(d) also covers degenerated sequences of the derivatives in 1(a) and 1(b).

For the above reasons, Claim 1 is not novel over D1-D3.

2.2 Claims 3-7, 11-20 and 22-25, which are dependent on Claim 1, are not novel either, in particular over D3.

D3 discloses the following in the abstract, the examples (in particular Example 3) and the claims (in particular Claims 1, 2, 6, 11, 21-28, 31 and 34):

- α-glucosidase from potatoes;
- constructs with promoters (including antisense promoters) cloned in vectors;
- introduction of the vectors into host cells (of vegetable or microbiological origin);
- transformation of potatoes;
- use of the sequence for producing modified starch for use in the foodstuff industry;
- modified starch.

2.3 The content of Claims 22 and 23 is unclear (see Box VIII) and not novel. Starch is a long-known substance with manifold uses. A wide variety of modified startches are also known. A product claim which defines the product only by a new preparation method is only possible when the product as such is novel and inventive.

Since, in addition, Claim 22 does not contain any technical features, a conclusive examination of this claim is not possible.

3. Inventive step (PCT Article 33(3)):

3.1 Claim 1 of the present application concerns a nucleic acid molecule that codes for an

α-glucosidase from potatoes. D1 discloses the sequence of an α-glucosidase from spinach. This sequence is 54% identical to another α-glucosidase sequence (the sequence in D2). Moreover, the sequence of the catalytic centre is known. That sequence is preserved to a high degree not only among plants, but also between animals and microorganisms. Knowledge of the two plant sequences and of the sequence of the catalytic centre is sufficient to allow a person skilled in the art to isolate the corresponding enzyme from other plant types also. Consequently, an inventive step cannot be recognised in the isolation of an α-glucosidase from potatoes.

3.2 Claims 2 and 8-10 concern the combination of α-glucosidase with other enzymes involved in starch metabolism, in particular starch synthesis. Documents D4-D7 all deal with the question of how to produce modified starch in plants by modifying the plant enzymes involved.

D4 discloses that modified starch can be produced by altering the equilibrium of the enzymes involved in starch biosynthesis (page 6, line 25). It is also noted that all the sequences known at the time can be used (page 7) and that the constructs can be used as sense or anti-sense constructs. It is expressly noted that more than one gene associated with the synthetic pathway can be modified (page 12).

D7 discloses an analogous teaching. On page 27 of D7, it is pointed out that structurally modified starch can be produced by increased or reduced

expression of the enzymes in question. It is expressly noted that any combination of enzymes is possible.

In the light of the documents mentioned, the combination of α-glucosidase with other known enzymes, as defined in Claims 2 and 8-10 of the present application, cannot be considered inventive.

3.3 Claim 21 likewise cannot be considered inventive, since all claims to which Claim 21 refers are either not novel or not inventive.

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The following points in the present application are unclear (PCT Article 6):

1. The expression "derivatives" in Claim 1 is unclear inasmuch as it does not define the extent to which the claimed sequence can deviate from seq. ID No. 2 and still be covered by the scope of protection, and also inasmuch as it does not indicate how long the sequence in question should be. According to PCT Article 6, **the claim must be clear in itself**. Moreover, in the case of the present application, the definition of the claimed derivatives in the description is also considered unclear. The definition on pages 5 ff. covers a plurality of derivatives, and it is not clear whether they all actually have the required function. In addition, it is stated on page 13, lines 1-3, that the numbering of the sequence elements is not binding, and therefore the derivatives, strictly speaking, must satisfy only the condition described on page 13, lines 3-4, according to which at least one section (of undefined length) displays a "significant match" with the sequence according to the invention. This wording is unclear.

It is also noted that the sequence disclosed in seq. ID No. 2 ends with threonine at position 682. It is therefore not clear why the list on page 7 of the description also contains amino acid residues numbered from 693 H to 832 R. Furthermore, some of the nucleotides indicated on page 11 do not match

VIII. Certain observations on the international application

those of said sequence identity number.

2. The term "parts" used in Claims 1, 2, 8 and 9 is unclear, since it does not indicate either the length or the function of the part in question. In particular in Claims 2, 8 and 9, the parts also include sequences without any function.

3. The terms "hybridise" and "specifically hybridise" in Claims 2 and 6 is unclear since the hybridisation conditions are not indicated and there is no characterisation of the hybridising molecule by its function. Moreover, the lengths of the hybridising sequences are not defined. For this reason, the present wording also covers sequences with a length of only a few nucleotides and having an entirely different function (or even none at all).

3.1 In particular, it is noted that a nucleic acid molecule of undefined length that hybridises with a nucleic acid molecule as per Claim 2 can bind to the sequence coding for the branching enzyme or a soluble starch synthase, for example. Since these sequences are part of the prior art, such nucleic acid sequences would not be novel.

4. The fact that a cell originates from another cell does not ensure that said cell also has all the properties of the parent cell. It is not clear from Claim 12 that the descendant cell must have the same functions as the parent cell.

5. Claim 18 concerns a starch-storing plant. Starch *per se* is a molecule which enables a plant to store sugars and to make them available to the metabolism

VIII. Certain observations on the international application

when required. Starch *per se* is therefore a storage substance. In addition, starch is produced by practically all plants and also stored for a certain time, so that it can fulfil its purpose (see above). For this reason, the expression "starch-storing plant" is unclear, since it does not allow a person skilled in the art to distinguish clearly which plants fall under the scope of protection and which do not. The list in the description (page 31 of the application) does not help to clarify this question, since this list also contains plants which are generally used for producing oils (e.g. rape, sunflower) or fibres (e.g. hemp, flax) and which therefore cannot be regarded as specifically starch-storing plants.

6. Claims 22 and 23 are unclear. They concern starch as a product and the use of this product, respectively. However, the product in Claim 22 is not characterised by any (technical) features, and therefore cannot be delimited from other similar products. In addition, the expression "can be obtained from" indicates only that the path presented is **one** possibility for producing starch and that the product in question can also be obtained in other ways. Consequently, a conclusive examination of the claims in question cannot be carried out, since the basis for the examination, namely the presence of product features, is lacking.

It is noted that starch as such is known from its everyday use and that, in addition, methods are known which make possible many modifications of

VIII. Certain observations on the international application

starch. For this reason, the claimed starch cannot be considered novel (see Box V).

It is also noted that the EPO, for example, recognises claims drafted as product-by-process claims only when the claimed product is novel and inventive.

Copy for the Elected Office (EO/US)

PCT/EP99/05536

.TENT COOPERATION TR' Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

AVENTIS CROPSCIENCE GMBH
Gebäude K 801
D-65926 Frankfurt am Main
ALLEMAGNE

Date of mailing (day/month/year)
14 April 2000 (14.04.00)

IMPORTANT NOTIFICATION

Applicant's or agent's file reference
1998/M225 PCT

International application No.
PCT/EP99/05536

International filing date (day/month/year)
30 July 1999 (30.07.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
HOECHST SCHERING AGREVO GMBH
Miraustrasse 54
D-13509 Berlin
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State of Nationality: DE
State of Residence: DE
Telephone No.: 069 305 82808
Facsimile No.: 069 305 2200
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
AVENTIS CROPSCIENCE GMBH
Miraustrasse 54
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Germany

State of Nationality: DE
State of Residence: DE
Telephone No.: 069 305 82808
Facsimile No.: 069 305 2200
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
N. Lindner

Telephone No.: (41-22) 338.83.38

Form PCT/IB/306 (March 1994)

003230137

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 09 March 2000 (09.03.00)	
International application No. PCT/EP99/05536	**Applicant's or agent's file reference** 1998/M225 PCT
International filing date (day/month/year) 30 July 1999 (30.07.99)	**Priority date** (day/month/year) 31 July 1998 (31.07.98)

Applicant

FROHBERG, Claus

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   29 January 2000 (29.01.00)

   [ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No.: (41-22) 740.14.35	Authorized officer Claudio Borton Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

To:

AVENTIS CROPSCIENCE GMBH
Gebäude K 801
D-65926 Frankfurt am Main
ALLEMAGNE

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

Date of mailing (day/month/year)
15 August 2000 (15.08.00)

Applicant's or agent's file reference
1998/M225 PCT

IMPORTANT NOTIFICATION

International application No.
PCT/EP99/05536

International filing date (day/month/year)
30 July 1999 (30.07.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

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Teleprinter No.:

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[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

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Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
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T1
16

VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS

PCT

INTERNATIONALER VORLÄUFIGER PRÜFUNGSBERICHT

(Artikel 36 und Regel 70 PCT)

Aktenzeichen des Anmelders oder Anwalts 1998/M225PCT	**WEITERES VORGEHEN** siehe Mitteilung über die Übersendung des internationalen vorläufigen Prüfungsbericht (Formblatt PCT/IPEA/416)	
Internationales Aktenzeichen PCT/EP99/05536	Internationales Anmeldedatum *(Tag/Monat/Jahr)* 30/07/1999	Prioritätsdatum *(Tag/Monat/Tag)* 31/07/1998
Internationale Patentklassification (IPK) oder nationale Klassifikation und IPK C12N15/55		
Anmelder AVENTIS CROPSCIENCE GMBH		

1. Dieser internationale vorläufige Prüfungsbericht wurde von der mit der internationale vorläufigen Prüfung beauftragte Behörde erstellt und wird dem Anmelder gemäß Artikel 36 übermittelt.

2. Dieser BERICHT umfaßt insgesamt 10 Blätter einschließlich dieses Deckblatts.

   ☐ Außerdem liegen dem Bericht ANLAGEN bei; dabei handelt es sich um Blätter mit Beschreibungen, Ansprüchen und/oder Zeichnungen, die geändert wurden und diesem Bericht zugrunde liegen, und/oder Blätter mit vor dieser Behörde vorgenommenen Berichtigungen (siehe Regel 70.16 und Abschnitt 607 der Verwaltungsrichtlinien zum PCT).

   Diese Anlagen umfassen insgesamt Blätter.

3. Dieser Bericht enthält Angaben zu folgenden Punkten:

   - I ☒ Grundlage des Berichts
   - II ☐ Priorität
   - III ☒ Keine Erstellung eines Gutachtens über Neuheit, erfinderische Tätigkeit und gewerbliche Anwendbarkeit
   - IV ☐ Mangelnde Einheitlichkeit der Erfindung
   - V ☒ Begründete Feststellung nach Artikel 35(2) hinsichtlich der Neuheit, der erfinderische Tätigkeit und der gewerbliche Anwendbarkeit; Unterlagen und Erklärungen zur Stützung dieser Feststellung
   - VI ☐ Bestimmte angeführte Unterlagen
   - VII ☐ Bestimmte Mängel der internationalen Anmeldung
   - VIII ☒ Bestimmte Bemerkungen zur internationalen Anmeldung

Datum der Einreichung des Antrags 29/01/2000	Datum der Fertigstellung dieses Berichts 12.10.2000
Name und Postanschrift der mit der internationalen vorläufigen Prüfung beauftragten Behörde: Europäisches Patentamt D-80298 München Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Bevollmächtigter Bediensteter Kurz, B Tel. Nr. +49 89 2399 7319

I. Grundlage des Berichts

1. Dieser Bericht wurde erstellt auf der Grundlage (*Ersatzblätter, die dem Anmeldeamt auf eine Aufforderung nach Artikel 14 hin vorgelegt wurden, gelten im Rahmen dieses Berichts als "ursprünglich eingereicht" und sind ihm nicht beigefügt, weil sie keine Änderungen enthalten.)*:

Beschreibung, Seiten:

1-58 ursprüngliche Fassung

Patentansprüche, Nr.:

1-25 ursprüngliche Fassung

Zeichnungen, Blätter:

1/1 ursprüngliche Fassung

2. Aufgrund der Änderungen sind folgende Unterlagen fortgefallen:

☐ Beschreibung, Seiten:

☐ Ansprüche, Nr.:

☐ Zeichnungen, Blatt:

3. ☐ Dieser Bericht ist ohne Berücksichtigung (von einigen) der Änderungen erstellt worden, da diese aus den angegebenen Gründen nach Auffassung der Behörde über den Offenbarungsgehalt in der ursprünglich eingereichten Fassung hinausgehen (Regel 70.2(c)):

4. Etwaige zusätzliche Bemerkungen:

siehe Beiblatt

III. Keine Erstellung eines Gutachtens über Neuheit, erfinderische Tätigkeit und gewerbliche Anwendbarkeit

Folgende Teile der Anmeldung wurden nicht daraufhin geprüft, ob die beanspruchte Erfindung als neu, auf erfinderischer Tätigkeit beruhend (nicht offensichtlich) und gewerblich anwendbar anzusehen ist:

☐ die gesamte internationale Anmeldung.

☒ Ansprüche Nr. 22, 23 (ganz); 2-4, 6, 7, 9-21, 24, 25 (teilweise).

Begründung:

☐ Die gesamte internationale Anmeldung, bzw. die obengenannten Ansprüche Nr. beziehen sich auf den nachstehenden Gegenstand, für den keine internationale vorläufige Prüfung durchgeführt werden braucht (*genaue Angaben*):

☒ Die Beschreibung, die Ansprüche oder die Zeichnungen (*machen Sie hierzu nachstehend genaue Angaben*) oder die obengenannten Ansprüche Nr. 22, 23 sind so unklar, daß kein sinnvolles Gutachten erstellt werden konnte (*genaue Angaben*):

siehe Beiblatt

☐ Die Ansprüche bzw. die obengenannten Ansprüche Nr. sind so unzureichend durch die Beschreibung gestützt, daß kein sinnvolles Gutachten erstellt werden konnte.

☒ Für die obengenannten Ansprüche Nr. 2-4, 6, 7, 9-21, 24, 25 (teilweise) wurde kein internationaler Recherchenbericht erstellt.

V. Begründete Feststellung nach Artikel 35(2) hinsichtlich der Neuheit, der erfinderischen Tätigkeit und der gewerblichen Anwendbarkeit; Unterlagen und Erklärungen zur Stützung dieser Feststellung

1. Feststellung

Neuheit (N)	Ja:	Ansprüche	2, 8-10, 21
	Nein:	Ansprüche	1, 3-7, 11-20, 22-25
Erfinderische Tätigkeit (ET)	Ja:	Ansprüche	-
	Nein:	Ansprüche	1-25
Gewerbliche Anwendbarkeit (GA)	Ja:	Ansprüche	1-25
	Nein:	Ansprüche	-

2. Unterlagen und Erklärungen

siehe Beiblatt

VIII. Bestimmte Bemerkungen zur internationalen Anmeldung

Zur Klarheit der Patentansprüche, der Beschreibung und der Zeichnungen oder zu der Frage, ob die Ansprüche in vollem Umfang durch die Beschreibung gestützt werden, ist folgendes zu bemerken:

siehe Beiblatt

Zu Punkt I
Grundlage des Berichtes

zu 4.:
Das Sequenzprotokoll mit den Seiten 1-5 war Teil der Anmeldeunterlagen und wurde in die Prüfung einbezogen.

Zu Punkt III
Keine Erstellung eines Gutachtens über Neuheit, erfinderische Tätigkeit und gewerbliche Anwendbarkeit

Die Prüfung der Ansprüche 2-4, 6, 7, und 9-25 erfolgte nur in dem Rahmen, der durch den Internationalen Recherchebericht abgedeckt wurde. Im vorliegenden Fall erfolgte die Prüfung der oben genannten Ansprüche also für die Kombination der Enzyme alpha-Glukosidase mit verschiedenen Stärkesynthasen (SS I, SS II, SS III) sowie mit Verzweigungsenzym (BE) wie im Recherchebericht dargelegt.
Die Beschreibung sowie die Formulierung der betreffenden Ansprüche entspricht daher im Moment nicht dem Prüfungsumfang.
Die Ansprüche 22 und 23 sind unklar (siehe Abschnitt VIII, 9.). Soweit möglich wird in Abschnitt V auf den Inhalt der Ansprüche eingegangen.

Zu Punkt V
Begründete Feststellung nach Regel 66.2(a)(ii) hinsichtlich der Neuheit, der erfinderischen Tätigkeit und der gewerblichen Anwendbarkeit; Unterlagen und Erklärungen zur Stützung dieser Feststellung

Es wird auf die folgenden Dokumente verwiesen:

D1: SUGIMOTO M. ET AL.: 'Molecular cloning and characterization of a cDNA encoding alpha-glucosidase from spinach' PLANT MOLECULAR BIOLOGY, Bd. 33, 1997, Seiten 765-768, in der Anmeldung erwähnt
D2: US-A-5 763 252 (TIBBOT BRIAN K ET AL) 9. Juni 1998 (1998-06-09)
D3: WO 97 24448 A (NICKERSON BIOCEM LTD; TAYLOR MARK ANDREW (GB); DAVIES HOWARD VIVIA) 10. Juli 1997 (1997-07-10)

D4: WO 94 09144 A (ZENECA LTD) 28. April 1994 (1994-04-28)
D5: WO 95 07355 A (INST GENBIOLOGISCHE FORSCHUNG; KOSSMANN JENS (DE); VIRGIN IVAR (DE) 16. März 1995 (1995-03-16), in der Anmeldung erwähnt
D6: WO 97 11188 A (KOSSMANN JENS; LORBERTH RUTH (DE); PLANTTEC BIOTECHNOLOGIE GMBH (D) 27. März 1997 (1997-03-27), in der Anmeldung erwähnt
D7: WO 96 15248 A (ABEL GERNOT J; INST GENBIOLOGISCHE FORSCHUNG (DE); KOSSMANN JENS (DE) 23. Mai 1996 (1996-05-23), in der Anmeldung erwähnt

Die vorliegende Anmeldung bezieht sich auf eine α-Glukosidase, die sie codierenden Nukleinsäuremoleküle und auf Kombinationen der α-Glukosidase mit anderen an der Stärkebildung beteiligten Enzymen.

1. Neuheit (Artikel 33(2) PCT):

1.1 Die Dokumente D1, D2 und D3 offenbaren jeweils eine aus verschiedenen Quellen isolierte α-Glukosidase. Die α-Glukosidase in D1 stammt aus Spinat und zeigt auf Aminosäureniveau 62% und auf Nukleinsäureniveau 68% Sequenzidentität. Das Enzym aus D2 wurde aus Gerste isoliert und zeigt sowohl auf Aminosäure- als auch auf Nukleotidniveau etwa 58% Sequenzidentität. Die in D3 offenbarte α-Glukosidase stammt aus Kartoffel und zeigt auf Aminosäureniveau 33% Identität mit dem Enzym der vorliegenden Anmeldung. Alle drei genannten Enzyme bzw. die sie codierenden Sequenzen erfüllen somit die Anforderung des Anspruchs 1c) bzw. 1d), d.h. sie stellen Sequenzen dar, die mit den unter 1a) und 1b) genannten Sequenzen hybridisieren. Die in D1-D3 offenbarten Sequenzen sind nicht identisch mit Seq ID1, können aber als Derivate bzw. als hybridisierende Sequenzen eingestuft werden, was durch unklare Formulierungen (siehe Abschnitt VIII, 1.-4.) zusätzlich begünstigt wird. Desweiteren ist das einzige Merkmal, mit dessen Hilfe ein Enzym charakterisiert werden kann, die Sequenz. Die Herkunft, in diesem Fall also die Isolation aus Kartoffel, stellt kein charakterisierendes Merkmal dar. Liegt das Enzym in gereinigter Form vor, kann der Fachmann vom Enzym selbst nicht auf die Quelle schließen, aus der es isoliert wurde.
Aus den oben genannten Gründen ist Anspruch 1 nicht neu gegenüber D1-D3.

1.2 Auch die von Anspruch 1 abhängigen Ansprüche 3-7, 11-20 und 22-25 sind insbesondere in bezug auf D3 nicht neu.

D3 offenbart in der Zusammenfassung, in den Beispielen (insbesondere Bsp. 3) und in den Ansprüchen (insbesondere Ansprüche 1, 2, 6, 11, 21-28, 31 und 34) folgendes:

- α-Glukosidase aus Kartoffel
- Konstrukte mit Promoter (auch antisense), die in Vektoren kloniert wurden
- Einführen der Vektoren in Wirtszellen (pflanzlichen oder mikrobiologischen Ursprungs)
- Transformation von Kartoffeln
- Benutzung der Sequenz zur Produktion modifizierter Stärke für die Anwendung im Nahrungsmittelbereich
- Modifizierte Stärke

1.3 Der Inhalt der Ansprüche 22 und 23 ist unklar (siehe VIII, 9.) und nicht neu. Stärke ist ein seit langem bekannter Stoff, der vielfältig verwendet wird. Auch in verschiedenster Weise modifizierte Stärke ist bereits bekannt. Ein Produktanspruch, der das Erzeugnis ausschließlich über eine neue Art der Herstellung definiert, ist nur dann möglich, wenn das Erzeugnis als solches neu und erfinderisch ist.
Da Anspruch 22 außerdem keine technischen Merkmale enthält, ist eine Prüfung dieses Anspruchs nicht abschließend möglich.

2. Erfinderische Tätigkeit (Artikel 33(3) PCT):

2.1 Sollte die Neuheit der unter 1. erwähnten Ansprüche wiederhergestellt werden, wird ihr Inhalt als nicht erfinderisch im Sinne von Artikel 33(3) PCT erachtet werden.
D1 offenbart die Sequenz einer α-Glukosidase aus Spinat. Diese Sequenz ist mit einer weiteren α-Glukosidasesequenz zu 54 % identisch. Außerdem ist die Sequenz des katalytischen Zentrums bekannt. Diese Sequenz ist nicht nur unter Pflanzen, sondern auch zwischen Tieren und Mikroorganismen hochgradig konserviert. Die Kenntnis der beiden pflanzlichen Sequenzen sowie der Sequenz des katalytischen Zentrums ist für einen Fachmann ausreichend, um das

entsprechende Enzym auch aus weiteren Pflanzenarten zu isolieren. Eine erfinderische Tätigkeit für die Isolierung einer α-Glukosidase aus Kartoffel kann somit nicht zuerkannt werden.

2.2 Die Ansprüche 2 und 8-10 beziehen sich auf die Kombination von α-Glukosidase mit anderen Enzymen, die am Stärkemetabolismus und insbesondere an der Stärkesynthese beteiligt sind. Die Dokumente D4-D7 beschäftigen sich alle mit der Frage, wie modifizierte Stärke durch eine Veränderung der beteiligten Enzyme in Pflanzen produziert werden kann.
D4 offenbart, daß veränderte Stärke durch eine Änderung des Gleichgewichts der an der Stärkebiosynthese beteiligten Enzyme hergestellt werden kann (Seite 6, Zeile 25). Weiterhin wird darauf hingewiesen, daß alle zur damaligen Zeit bekannten Sequenzen verwendet werden können (Seite 7) und daß die Konstrukte in Sense- oder Antisenseorientierung verwendet werden können. Es wird ausdrücklich darauf verwiesen, daß mehr als ein Gen des Syntheseweges verändert werden kann (Seite 12).
Eine analoge Lehre wird in D7 offenbart. Auf Seite 27 von D7 wird darauf hingewiesen, daß strukturell veränderte Stärke durch erhöhte oder verminderte Expression der betreffenden Enzyme hergestellt werden kann. Es wird ausdrücklich darauf hingewiesen, daß jede Kombination der Enzyme möglich ist.

In bezug auf die erwähnten Dokumente kann die Kombination von α-Glukosidase mit anderen bekannten Enzymen, wie in den Ansprüchen 2 und 8-10 der aktuellen Anmeldung dargestellt, nicht als erfinderisch anerkannt werden.

2.3 Anspruch 21 kann ebenfalls nicht als erfinderisch anerkannt werden, da alle Ansprüche, auf die sich Anspruch 21 bezieht, entweder nicht neu oder nicht erfinderisch sind.

Zu Punkt VIII

Bestimmte Bemerkungen zur internationalen Anmeldung

Folgende Punkte der vorliegenden Anmeldung sind unklar (Artikel 6 PCT):

1. Der Ausdruck "Derivate" in Anspruch 1 ist dahingehend unklar, als er offen läßt, in welchem Maß die beanspruchte Sequenz von Seq. ID 2 abweichen kann, um noch unter den Schutzumfang zu fallen und wie lang die betreffende Sequenz sein soll. Außerdem ist unklar, ob das Derivat dieselbe Funktion hat wie das von Seq. ID 2 codierte Protein.
2. Der in den Ansprüchen 1, 2, 8 und 9 verwendete Ausdruck "Teile" ist unklar, da aus ihm weder die Länge noch die Funktion des jeweiligen Teiles hervorgeht.

3. Die Formulierungen "hybridisieren" bzw. "spezifisch hybridisieren" in den Ansprüchen 1, 2 und 6 sind unklar, da weder Hybridisierungsbedingungen genannt werden, noch eine Charakterisierung des hybridisierenden Moleküls durch seine Funktion vorliegt. Desweiteren ist die Länge der hybrisdisierenden Sequenzen nicht definiert. Aus diesem Grund sind in der aktuellen Formulierung auch Sequenzen eingeschlossen, die eine Länge von wenigen Basenpaaren umfassen und die eine völlig andere (oder sogar gar keine) Funktion aufweisen können.

4. Anspruch 1d) ist unklar, da aus ihm nicht hervorgeht, daß die degenerierte Sequenz die Funktion einer α-Glukosidase haben muß. Die vorliegende Formulierung umfaßt auch Sequenzen mit einer anderen bzw. ohne jegliche Funktion. Dies gilt insbesondere, da sich Anspruch 1d) auf 1c) rückbezieht und die Funktion der in 1c) beanspruchten Nukleinsäuremoleküle bereits unklar ist (siehe 3.).

5. Die Formulierung von Anspruch 10 ist unpräzise. Es ist nicht klar, wie eine Nukleotidsequenz, die für **ein** Protein codiert (also z.B. eine Sequenz gemäß Anspruch 1), teilweise in sense- und teilweise in antisense-Richtung vorliegen kann. Außerdem ist ein Protein der Gruppe A in Anspruch 1 nicht erwähnt, so daß sich Anspruch 10 in der aktuellen Formulierung höchstens auf die Ansprüche 2-6 beziehen kann.

6. Die Tatsache, daß eine Zelle von einer anderen Zelle abstammt, gewährleistet nicht, daß besagte Zelle auch sämtliche Eigenschaften der Elternzelle aufweist. Aus Anspruch 12 geht nicht hervor, daß die abgestammte Zelle identische Funktionen wie die Elternzelle aufweisen muß.

7. Die Formulierung von Anspruch 16 kann so verstanden werden, daß die beanspruchte Pflanze nur eine einzige transformierte Pflanzenzelle enthalten soll. Aus dem aktuellen Wortlaut geht nicht hervor, daß die beanspruchte Pflanze mehr als eine transformierte Zelle enthalten soll oder kann.

8. Anspruch 18 bezieht sich auf eine stärkespeichernde Pflanze. Stärke an sich ist ein Molekül, das der Pflanze die Speicherung von Zuckerstoffen erlaubt, um sie bei Bedarf für den Stoffwechsel zur Verfügung zu stellen. Stärke per se ist also ein Speicherstoff. Noch dazu wird Stärke von praktisch allen Pflanzen produziert und auch über eine gewisse Zeit gelagert, damit sie ihren Zweck (siehe oben) erfüllen kann. Aus diesem Grund ist der Ausdruck "stärkespeichernde Pflanze" unklar, da er es dem Fachmann nicht ermöglicht, klar zu unterscheiden, welche Pflanzen unter den Schutzumfang des Anspruchs fallen und welche nicht.
Auch die Aufzählung in der Beschreibung (S. 31 der Anmeldung) trägt nicht zur Klärung bei, da diese Aufzählung auch Pflanzen enthält, die im allgemeinen zur Ölproduktion (z.B. Raps, Sonnenblume) oder zur Faserproduktion (z.B. Hanf, Flachs) verwendet werden und somit keineswegs als speziell stärkespeichernd angesehen werden können.

9. Die Ansprüche 22 und 23 sind unklar. Sie beziehen sich auf Stärke als Produkt bzw. auf die Verwendung dieses Produktes. Das Produkt in Anspruch 22 ist jedoch durch keinerlei (technische) Merkmale charakterisiert, so daß es nicht von anderen, ähnlichen Produkten abgegrenzt werden kann. Desweiteren sagt der Begriff "erhältlich aus" lediglich aus, daß der dargestellte Weg eine Möglichkeit zur Produktion der Stärke ist und das betreffende Produkt auch anders erhalten werden kann. Infolgedessen kann für die betreffenden Ansprüche keine Prüfung durchgeführt werden, da die Basis für die Prüfung, d.h. das Vorhandensein von Produktmerkmalen, fehlt.
Stärke als solche ist aus dem Alltagsgebrauch bekannt und somit nicht neu (siehe Abschnitt V).

10. In der Beschreibung auf den Seiten 5-7 wird ein Derivat beschrieben, dessen Aminosäuresequenz bestimmte Aminosäurereste gemäß der Auflistung enthalten soll. Es wird ausdrücklich erwähnt (Seite 7, Zeile 23), daß die Aminosäurereste aus Seq ID 2 ausgewählt sind. Da die in Seq. ID 2 offenbarte Sequenz mit Threonin auf Position 682 endet, ist nicht klar, warum die Auflistung auch Aminosäurereste mit einer Numerierung von 693 H bis 832 R beinhaltet.

10

VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS

PCT

INTERNATIONALER VORLÄUFIGER PRÜFUNGSBERICHT

(Artikel 36 und Regel 70 PCT)

T 16

Aktenzeichen des Anmelders oder Anwalts 1998/M225PCT	**WEITERES VORGEHEN** siehe Mitteilung über die Übersendung des internationalen vorläufigen Prüfungsbericht (Formblatt PCT/IPEA/416)	
Internationales Aktenzeichen PCT/EP99/05536	Internationales Anmeldedatum *(Tag/Monat/Jahr)* 30/07/1999	Prioritätsdatum *(Tag/Monat/Tag)* 31/07/1998
Internationale Patentklassification (IPK) oder nationale Klassifikation und IPK C12N15/55		
Anmelder AVENTIS CROPSCIENCE GMBH		

1. Dieser internationale vorläufige Prüfungsbericht wurde von der mit der internationale vorläufigen Prüfung beauftragte Behörde erstellt und wird dem Anmelder gemäß Artikel 36 übermittelt.

2. Dieser BERICHT umfaßt insgesamt 11 Blätter einschließlich dieses Deckblatts.

   ☒ Außerdem liegen dem Bericht ANLAGEN bei; dabei handelt es sich um Blätter mit Beschreibungen, Ansprüchen und/oder Zeichnungen, die geändert wurden und diesem Bericht zugrunde liegen, und/oder Blätter mit vor dieser Behörde vorgenommenen Berichtigungen (siehe Regel 70.16 und Abschnitt 607 der Verwaltungsrichtlinien zum PCT).

   Diese Anlagen umfassen insgesamt 2 Blätter.

CORRECTED VERSION

3. Dieser Bericht enthält Angaben zu folgenden Punkten:

   - I ☒ Grundlage des Berichts
   - II ☐ Priorität
   - III ☒ Keine Erstellung eines Gutachtens über Neuheit, erfinderische Tätigkeit und gewerbliche Anwendbarkeit
   - IV ☐ Mangelnde Einheitlichkeit der Erfindung
   - V ☒ Begründete Feststellung nach Artikel 35(2) hinsichtlich der Neuheit, der erfinderische Tätigkeit und der gewerbliche Anwendbarkeit; Unterlagen und Erklärungen zur Stützung dieser Feststellung
   - VI ☐ Bestimmte angeführte Unterlagen
   - VII ☐ Bestimmte Mängel der internationalen Anmeldung
   - VIII ☒ Bestimmte Bemerkungen zur internationalen Anmeldung

Datum der Einreichung des Antrags 29/01/2000	Datum der Fertigstellung dieses Berichts 23.11.2000
Name und Postanschrift der mit der internationalen vorläufigen Prüfung beauftragten Behörde: Europäisches Patentamt D-80298 München Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Bevollmächtigter Bediensteter Kurz, B Tel. Nr. +49 89 2399 7319

INTERNATIONALER VORLÄUFIGER PRÜFUNGSBERICHT

Internationales Aktenzeichen PCT/EP99/05536

I. Grundlage des Berichts

1. Dieser Bericht wurde erstellt auf der Grundlage (*Ersatzblätter, die dem Anmeldeamt auf eine Aufforderung nach Artikel 14 hin vorgelegt wurden, gelten im Rahmen dieses Berichts als "ursprünglich eingereicht" und sind ihm nicht beigefügt, weil sie keine Änderungen enthalten.)*:
Beschreibung, Seiten:

1-58 ursprüngliche Fassung

Patentansprüche, Nr.:

12-25 ursprüngliche Fassung

1-11 mit Telefax vom 13/11/2000

Zeichnungen, Blätter:

1/1 ursprüngliche Fassung

Sequenzprotokoll in der Beschreibung, Seiten:

1-5, in der ursprünglich eingereichten Fassung.

2. Hinsichtlich der **Sprache**: Alle vorstehend genannten Bestandteile standen der Behörde in der Sprache, in der die internationale Anmeldung eingereicht worden ist, zur Verfügung oder wurden in dieser eingereicht, sofern unter diesem Punkt nichts anderes angegeben ist.

   Die Bestandteile standen Behörde in der Sprache: , zur Verfügung bzw. wurden in dieser Sprache eingereicht; dabei handelt es sich um

   ☐ die Sprache der Übersetzung, die für die Zwecke der internationalen Recherche eingereicht worden ist (nach Regel 23.1(b)).

   ☐ die Veröffentlichungssprache der internationalen Anmeldung (nach Regel 48.3(b)).

   ☐ die Sprache der Übersetzung, die für die Zwecke der internationalen vorläufigen Prüfung eingereicht worden ist (nach Regel 55.2 und/oder 55.3).

3. Hinsichtlich der in der internationalen Anmeldung offenbarten **Nucleotid- und/oder Aminosäuresequenz** ist die internationale vorläufige Prüfung auf der Grundlage des Sequenzprotokolls durchgeführt worden, das:

   ☐ in der internationalen Anmeldung in schriftlicher Form enthalten ist.

   ☒ zusammen mit der internationalen Anmeldung in computerlesbarer Form eingereicht worden ist.

   ☐ bei der Behörde nachträglich in schriftlicher Form eingereicht worden ist.

   ☐ bei der Behörde nachträglich in computerlesbarer Form eingereicht worden ist.

   ☐ Die Erklärung, dass das nachträglich eingereichte schriftliche Sequenzprotokoll nicht über den

Offenbarungsgehalt der internationalen Anmeldung im Anmeldezeitpunkt hinausgeht, wurde vorgelegt.

☐ Die Erklärung, dass die in computerlesbarer Form erfassten Informationen dem schriftlichen Sequenzprotokoll entsprechen, wurde vorgelegt.

4. Aufgrund der Änderungen sind folgende Unterlagen fortgefallen:

☐ Beschreibung, Seiten:

☐ Ansprüche, Nr.:

☐ Zeichnungen, Blatt:

5. ☐ Dieser Bericht ist ohne Berücksichtigung (von einigen) der Änderungen erstellt worden, da diese aus den angegebenen Gründen nach Auffassung der Behörde über den Offenbarungsgehalt in der ursprünglich eingereichten Fassung hinausgehen (Regel 70.2(c)).

(Auf Ersatzblätter, die solche Änderungen enthalten, ist unter Punkt 1 hinzuweisen;sie sind diesem Bericht beizufügen).

6. Etwaige zusätzliche Bemerkungen:

III. Keine Erstellung eines Gutachtens über Neuheit, erfinderische Tätigkeit und gewerbliche Anwendbarkeit

Folgende Teile der Anmeldung wurden nicht daraufhin geprüft, ob die beanspruchte Erfindung als neu, auf erfinderischer Tätigkeit beruhend (nicht offensichtlich) und gewerblich anwendbar anzusehen ist:

☐ die gesamte internationale Anmeldung.

☒ Ansprüche Nr. 2-4, 6, 7, 9-25.

Begründung:

☐ Die gesamte internationale Anmeldung, bzw. die obengenannten Ansprüche Nr. beziehen sich auf den nachstehenden Gegenstand, für den keine internationale vorläufige Prüfung durchgeführt werden braucht (*genaue Angaben*):

☒ Die Beschreibung, die Ansprüche oder die Zeichnungen (*machen Sie hierzu nachstehend genaue Angaben*) oder die obengenannten Ansprüche Nr. 22, 23 sind so unklar, daß kein sinnvolles Gutachten erstellt werden konnte (*genaue Angaben*):
siehe Beiblatt

☐ Die Ansprüche bzw. die obengenannten Ansprüche Nr. sind so unzureichend durch die Beschreibung gestützt, daß kein sinnvolles Gutachten erstellt werden konnte.

☒ Für die obengenannten Ansprüche Nr. 2-4, 6, 7, 9-25 wurde kein internationaler Recherchenbericht erstellt.

2. Eine sinnvolle internationale vorläufige Prüfung kann nicht durchgeführt werden, weil das Protokoll der Nukleotid- und/oder Aminosäuresequenzen nicht dem in Anlage C der Verwaltungsvorschriften vorgeschriebenen Standard entspricht:

☐ Die schriftliche Form wurde nicht eingereicht bzw. entspricht nicht dem Standard.

☐ Die computerlesbare Form wurde nicht eingereicht bzw. entspricht nicht dem Standard.

V. Begründete Feststellung nach Artikel 35(2) hinsichtlich der Neuheit, der erfinderischen Tätigkeit und der gewerblichen Anwendbarkeit; Unterlagen und Erklärungen zur Stützung dieser Feststellung

1. Feststellung

Neuheit (N)	Ja:	Ansprüche	2, 8-10, 21
	Nein:	Ansprüche	1, 3-7, 11-20, 22-25
Erfinderische Tätigkeit (ET)	Ja:	Ansprüche	
	Nein:	Ansprüche	1-25
Gewerbliche Anwendbarkeit (GA)	Ja:	Ansprüche	1-25
	Nein:	Ansprüche	

2. Unterlagen und Erklärungen
siehe Beiblatt

VIII. Bestimmte Bemerkungen zur internationalen Anmeldung

Zur Klarheit der Patentansprüche, der Beschreibung und der Zeichnungen oder zu der Frage, ob die Ansprüche in vollem Umfang durch die Beschreibung gestützt werden, ist folgendes zu bemerken:
siehe Beiblatt

Zu Punkt I

Grundlage des Berichts

Das Sequenzprotokoll mit den Seiten 1-5 war Teil der Anmeldeunterlagen und wurde in die Prüfung einbezogen.

Zu Punkt III

Keine Erstellung eines Gutachtens über Neuheit, erfinderische Tätigkeit und gewerbliche Anwendbarkeit

1. Die Prüfung der Ansprüche 2-4, 6, 7 und 9-25 erfolgte nur in dem Rahmen, der durch den Internationalen Recherchenbericht abgedeckt wurde (Regel 66.1 e) PCT). Im vorliegenden Fall erfolgte die Prüfung der oben genannten Ansprüche für das Enzym α-Glukosidase sowie die Kombination von α-Glukosidase mit verschiedenen löslichen Stärkesynthasen (SS I, SS II, SS III) sowie mit Verzweigungsenzym (BE). Wie im Recherchenbericht dargelegt, basierte die Recherche auf denjenigen Nukleinsäuremolekülen, Verfahren und Pflanzen, die im Sinne von Artikel 6 PCT als von der Beschreibung gestützt und im Sinne von Artikel 5 PCT als ausreichend offenbart gelten können. Dies sind im vorliegenden Fall die in den Ausführungsbeispielen 1-12 dargelegten Nukleinsäuren, Verfahren und Pflanzen.
   Die Prüfung von Anmeldegegenständen, die nicht recherchiert wurden, ist nicht möglich.

2. Die Ansprüche 22 und 23 sind unklar (siehe Abschnitt VIII) und enthalten keine technischen Merkmale. Soweit möglich wird in den Abschnitten V und VIII auf den Inhalt der Ansprüche eingegangen. Aufgrund der fehlenden technischen Merkmale ist eine abschließende Prüfung jedoch nicht möglich.

Zu Punkt V

Begründete Feststellung nach Artikel 35(2) hinsichtlich der Neuheit, der erfinderischen Tätigkeit und der gewerblichen Anwendbarkeit; Unterlagen und Erklärungen zur Stützung dieser Feststellung

Es wird auf die folgenden Dokumente verwiesen:

D1: SUGIMOTO M. ET AL.: 'Molecular cloning and characterization of a cDNA encoding alpha-glucosidase from spinach' PLANT MOLECULAR BIOLOGY, Bd. 33, 1997, Seiten 765-768, in der Anmeldung erwähnt

D2: US-A-5 763 252 (TIBBOT BRIAN K ET AL) 9. Juni 1998 (1998-06-09)

D3: WO 97 24448 A (NICKERSON BIOCEM LTD; TAYLOR MARK ANDREW (GB); DAVIES HOWARD VIVIA) 10. Juli 1997 (1997-07-10)

D4: WO 94 09144 A (ZENECA LTD) 28. April 1994 (1994-04-28)

D5: WO 95 07355 A (INST GENBIOLOGISCHE FORSCHUNG; KOSSMANN JENS (DE); VIRGIN IVAR (DE) 16. März 1995 (1995-03-16), in der Anmeldung erwähnt

D6: WO 97 11188 A (KOSSMANN JENS; LORBERTH RUTH (DE); PLANTTEC BIOTECHNOLOGIE GMBH (D) 27. März 1997 (1997-03-27), in der Anmeldung erwähnt

D7: WO 96 15248 A (ABEL GERNOT J; INST GENBIOLOGISCHE FORSCHUNG (DE); KOSSMANN JENS (DE) 23. Mai 1996 (1996-05-23), in der Anmeldung erwähnt

Die vorliegende Anmeldung bezieht sich auf eine α-Glukosidase, die sie kodierenden Nukleinsäuremoleküle und auf Kombinationen der α-Glukosidase mit anderen an der Stärkebildung beteiligten Enzymen.

1. Die am 13.11.2000 per Fax eingereichten Änderungen entsprechen Artikel 19(2) und 34(2)b) PCT und sind somit zulässig.

2. Neuheit (Artikel 33(2) PCT):

2.1 Die Dokumente D1, D2 und D3 offenbaren jeweils eine aus verschiedenen Quellen isolierte α-Glukosidase. Die α-Glukosidase in D1 stammt aus Spinat und zeigt auf Aminosäureniveau 62% und auf Nukleinsäureniveau 68% Sequenzidentität. Das Enzym aus D2 wurde aus Gerste isoliert und zeigt sowohl auf Aminosäure- als auch auf Nukleotidniveau etwa 58% Sequenzidentität. Die in D3 offenbarte α-Glukosidase stammt aus Kartoffel und zeigt auf Aminosäureniveau 33% Identität mit dem Enzym der vorliegenden Anmeldung. Die in D1-D3 offenbarten Sequenzen sind nicht identisch mit Seq ID Nr. 1, können

aber als Derivate mit Funktion einer α-Glukosidase eingestuft werden, was durch unklare Formulierungen (siehe Abschnitt VIII) zusätzlich begünstigt wird. Alle drei genannten Enzyme bzw. die sie codierenden Sequenzen erfüllen somit die Anforderung des Anspruchs 1a) bzw. 1d).
Es wird darauf hingewiesen, daß die aktuelle Formulierung von Anspruch 1d) auch degenerierte Sequenzen der Derivate aus 1a) und 1b) umfaßt.

Aus den oben genannten Gründen ist Anspruch 1 nicht neu gegenüber D1-D3.

2.2 Auch die von Anspruch 1 abhängigen Ansprüche 3-7, 11-20 und 22-25 sind insbesondere in bezug auf D3 nicht neu.

D3 offenbart in der Zusammenfassung, in den Beispielen (insbesondere Bsp. 3) und in den Ansprüchen (insbesondere Ansprüche 1, 2, 6, 11, 21-28, 31 und 34) folgendes:
- α-Glukosidase aus Kartoffel
- Konstrukte mit Promoter (auch antisense), die in Vektoren kloniert wurden
- Einführen der Vektoren in Wirtszellen (pflanzlichen oder mikrobiologischen Ursprungs)
- Transformation von Kartoffeln
- Benutzung der Sequenz zur Produktion modifizierter Stärke für die Anwendung im Nahrungsmittelbereich
- Modifizierte Stärke

2.3 Der Inhalt der Ansprüche 22 und 23 ist unklar (siehe Abschnitt VIII) und nicht neu.
Stärke ist ein seit langem bekannter Stoff, der vielfältig verwendet wird. Auch in verschiedenster Weise modifizierte Stärke ist bereits bekannt. Ein Produktanspruch, der das Erzeugnis ausschließlich über eine neue Art der Herstellung definiert, ist nur dann möglich, wenn das Erzeugnis als solches neu und erfinderisch ist.
Da Anspruch 22 außerdem keine technischen Merkmale enthält, ist eine Prüfung dieses Anspruchs nicht abschließend möglich.

3. Erfinderische Tätigkeit (Artik I 33(3) PCT):

3.1 Anspruch 1 der vorliegenden Anmeldung bezieht sich auf ein Nukleinsäuremolekül, das für eine α-Glukosidaseaus aus Kartoffel kodiert. D1 offenbart die Sequenz einer α-Glukosidase aus Spinat. Diese Sequenz ist mit einer weiteren α-Glukosidasesequenz (der Sequenz aus D2) zu 54 % identisch. Außerdem ist die Sequenz des katalytischen Zentrums bekannt. Diese Sequenz ist nicht nur unter Pflanzen, sondern auch zwischen Tieren und Mikroorganismen hochgradig konserviert. Die Kenntnis der beiden pflanzlichen Sequenzen sowie der Sequenz des katalytischen Zentrums ist für einen Fachmann ausreichend, um das entsprechende Enzym auch aus weiteren Pflanzenarten zu isolieren. Eine erfinderische Tätigkeit für die Isolierung einer α-Glukosidase aus Kartoffel kann somit nicht zuerkannt werden.

3.2 Die Ansprüche 2 und 8-10 beziehen sich auf die Kombination von α-Glukosidase mit anderen Enzymen, die am Stärkemetabolismus und insbesondere an der Stärkesynthese beteiligt sind. Die Dokumente D4-D7 beschäftigen sich alle mit der Frage, wie modifizierte Stärke durch eine Veränderung der beteiligten Enzyme in Pflanzen produziert werden kann.
D4 offenbart, daß veränderte Stärke durch eine Änderung des Gleichgewichts der an der Stärkebiosynthese beteiligten Enzyme hergestellt werden kann (Seite 6, Zeile 25). Weiterhin wird darauf hingewiesen, daß alle zur damaligen Zeit bekannten Sequenzen verwendet werden können (Seite 7) und daß die Konstrukte in Sense- oder Antisenseorientierung verwendet werden können. Es wird ausdrücklich darauf verwiesen, daß mehr als ein Gen des Syntheseweges verändert werden kann (Seite 12).
Eine analoge Lehre wird in D7 offenbart. Auf Seite 27 von D7 wird darauf hingewiesen, daß strukturell veränderte Stärke durch erhöhte oder verminderte Expression der betreffenden Enzyme hergestellt werden kann. Es wird ausdrücklich darauf hingewiesen, daß jede Kombination der Enzyme möglich ist.

In bezug auf die erwähnten Dokumente kann die Kombination von α-Glukosidase mit anderen bekannten Enzymen, wie in den Ansprüchen 2 und 8-10 der aktuellen Anmeldung dargestellt, nicht als erfinderisch anerkannt werden.

3.3 Anspruch 21 kann ebenfalls nicht als erfinderisch anerkannt werden, da alle Ansprüche, auf die sich Anspruch 21 bezieht, entweder nicht neu oder nicht erfinderisch sind.

Zu Punkt VIII

Bestimmte Bemerkungen zur internationalen Anmeldung

Folgende Punkte der vorliegenden Anmeldung sind unklar (Artikel 6 PCT):

1. Der Ausdruck "Derivate" in Anspruch 1 ist dahingehend unklar, als er offen läßt, in welchem Maß die beanspruchte Sequenz von Seq. ID 2 abweichen kann, um noch unter den Schutzumfang zu fallen und wie lang die betreffende Sequenz sein soll. Gemäß Artikel 6 muß **der Anspruch an sich** klar sein.
   Im Fall der vorliegenden Anmeldung wird außerdem auch die Definition der beanspruchten Derivate in der Beschreibung als unklar angesehen. Die Definition auf Seite 5 ff umfaßt eine Vielzahl von Derivaten, von denen nicht klar ist, ob wirklich alle die geforderte Funktion haben. Desweiteren wird auf Seite 13, Zeile 1-3, dargelegt, daß die Numerierung der Sequenzelemente nicht bindend ist, so daß die Derivate genau genommen nur die auf Seite 13 Zeile 3-4 dargelegte Bedingung erfüllen müssen, daß mindestens ein Teilabschnitt (nicht definierter Länge) eine "signifikante Übereinstimmung" mit der erfindungsgemäßen Sequenz aufweist. Diese Formulierung ist unklar.
   Es wird desweiteren darauf hingewiesen, daß die in Seq. ID 2 offenbarte Sequenz mit Threonin auf Position 682 endet. Es ist daher nicht klar, warum die Auflistung auf Seite 7 der Beschreibung auch Aminosäurereste mit einer Numerierung von 693 H bis 832 R beinhaltet. Auch einige der auf Seite 11 genannten Nukleotide stimmen nicht mit denjenigen der genannten Seq. ID Nummer überein.

2. Der in den Ansprüchen 1, 2, 8 und 9 verwendete Ausdruck "Teile" ist unklar, da aus ihm weder die Länge noch die Funktion des jeweiligen Teiles hervorgeht. Insbesondere in den Ansprüchen 2, 8 und 9 umfassen die Teile auch Sequenzen ohne jegliche Funktion.

3. Die Formulierungen "hybridisieren" bzw. "spezifisch hybridisieren" in den Ansprüchen 2 und 6 sind unklar, da weder Hybridisierungsbedingungen genannt

werden, noch eine Charakterisierung des hybridisierenden Moleküls durch seine Funktion vorliegt. Desweiteren ist die Länge der hybrisdisierenden Sequenzen nicht definiert. Aus diesem Grund sind in der aktuellen Formulierung auch Sequenzen eingeschlossen, die eine Länge von wenigen Nukleotiden umfassen und die eine völlig andere (oder sogar gar keine) Funktion aufweisen können.

3.1 Es wird insbesondere darauf hingewiesen, daß ein Nukleinsäuremolekül nicht definierter Länge, das mit einem Nukleinsäuremolekül des Anspruchs 2 hybridisiert, z.B. an die für Verzweigungsenzym oder eine lösliche Stärkesynthase codierende Sequenz binden kann. Da diese Sequenzen zum Stand der Technik gehören, wären solche Nukleinsäuresequenzen nicht neu.

4. Die Tatsache, daß eine Zelle von einer anderen Zelle abstammt, gewährleistet nicht, daß besagte Zelle auch sämtliche Eigenschaften der Elternzelle aufweist. Aus Anspruch 12 geht nicht hervor, daß die abgestammte Zelle identische Funktionen wie die Elternzelle aufweisen muß.

5. Anspruch 18 bezieht sich auf eine stärkespeichernde Pflanze. Stärke an sich ist ein Molekül, das der Pflanze die Speicherung von Zuckerstoffen erlaubt, um sie bei Bedarf für den Stoffwechsel zur Verfügung zu stellen. Stärke per se ist also ein Speicherstoff. Noch dazu wird Stärke von praktisch allen Pflanzen produziert und auch über eine gewisse Zeit gelagert, damit sie ihren Zweck (siehe oben) erfüllen kann. Aus diesem Grund ist der Ausdruck "stärkespeichernde Pflanze" unklar, da er es dem Fachmann nicht ermöglicht, klar zu unterscheiden, welche Pflanzen unter den Schutzumfang des Anspruchs fallen und welche nicht. Auch die Aufzählung in der Beschreibung (S. 31 der Anmeldung) trägt nicht zur Klärung bei, da diese Aufzählung auch Pflanzen enthält, die im allgemeinen zur Ölproduktion (z.B. Raps, Sonnenblume) oder zur Faserproduktion (z.B. Hanf, Flachs) verwendet werden und somit keineswegs als speziell stärkespeichernd angesehen werden können.

6. Die Ansprüche 22 und 23 sind unklar. Sie beziehen sich auf Stärke als Produkt bzw. auf die Verwendung dieses Produktes. Das Produkt in Anspruch 22 ist jedoch durch keinerlei (technische) Merkmale charakterisiert, so daß es nicht von anderen, ähnlichen Produkten abgegrenzt werden kann. Desweiteren sagt der Begriff "erhältlich aus" lediglich aus, daß der dargestellte Weg **ein** Möglichkeit

zur Produktion der Stärke ist und das betreffende Produkt auch anders erhalten werden kann. Infolgedessen kann für die betreffenden Ansprüche keine abschließende Prüfung durchgeführt werden, da die Basis für die Prüfung, d.h. das Vorhandensein von Produktmerkmalen, fehlt.

Es wird darauf verwiesen, daß Stärke als solche aus dem Alltagsgebrauch bekannt ist und daß außerdem Methoden bekannt sind, die vielfältige Veränderungen von Stärke ermöglichen. Aus diesem Grund ist die beanspruchte Stärke als nicht neu anzusehen (siehe Abschnitt V).

Es wird weiterhin darauf hingewiesen, daß z.B. das EPA Ansprüche, die in Form eines Product-by-Process formuliert sind, nur anerkennt, wenn das beanspruchte Produkt neu und erfinderisch ist.

Patentansprüche:

1. Nukleinsäuremolekül, codierend ein Protein mit der Funktion einer α-Glukosidase aus Kartoffel, ausgewählt aus der Gruppe bestehend aus
a) Nukleinsäuremolekülen, die ein Protein codieren, das die unter Seq ID NO. 2 angegebene Aminosäuresequenz umfaßt, deren Derivate oder Teile;
b) Nukleinsäuremolekülen, die die unter Seq ID No. 1 dargestellte Nucleotidsequenz oder deren Derivate oder Teile umfassen oder eine korrespondierende Ribonucleotidsequenz;
c) Nukleinsäuremoleküle, die mit den unter (a) oder (b) genannten Nukleinsäuremolekülen spezifisch hybridisieren oder komplementär sind und eine Homologie von über 70% aufweisen, und
d) Nukleinsäuremolekülen, deren Nucleotidsequenz aufgrund der Degeneration des genetischen Codes von der Sequenz der unter (a), (b) oder (c) genannten Nukleinsäuremoleküle abweicht.

2. Rekombinantes Nukleinsäuremolekül, enthaltend
a) ein Nukleinsäuremolekül codierend für ein Protein mit der Funktion einer α-Glukosidase aus Kartoffel gemäß Anspruch 1 und
b) ein oder mehrere Nukleotidsequenzen, die für ein Protein kodieren, ausgewählt aus der Gruppe A, bestehend aus Proteinen mit der Funktion von Verzweigungsenzymen, ADP-Glukose-Pyrophosphorylasen, Stärkekorn-gebundenen Stärkesynthasen, löslichen Stärkesynthasen, Entzweigungsenzymen, Disproportionierungsenzymen, plastidären Stärkephosphorylasen, R1- Enzymen, Amylasen, Glukosidasen, Teilen besagter Nukleotidsequenzen oder mit besagten Nukleotidsequenzen hybridisierende Nukleinsäuremoleküle.

3. Nukleinsäuremolekül nach Anspruch 1 oder 2, das ein Desoxyribonukleinsäure-Molekül ist.

GEÄNDERTES BLATT
AMENDED SHEET

4. Nukleinsäuremolekül nach Anspruch 2, das ein cDNA-Molekül ist.

5. Nukleinsäuremolekül nach Anspruch 1, das ein Ribonukleinsäure-Molekül ist.

6. Nukleinsäuremolekül, das mit einem Nukleinsäuremolekül nach einem oder mehreren der Ansprüche 1 bis 5 spezifisch hybridisiert.

7. Vektor, enthaltend ein Nukleinsäuremolekül nach einem oder mehreren der Ansprüche 1 bis 6.

8. Vektor, enthaltend ein Nukleinsäuremolekül nach einem oder mehreren der Ansprüche 1-6, dadurch gekennzeichnet, daß die Nukleotidsequenz codierend für ein Protein mit der Funktion einer α-Glukosidase oder Teile davon in sense- oder anti-sense-Richtung vorliegt.

9. Vektor, enthaltend ein Nukleinsäuremolekül nach einem oder mehreren der Ansprüche 2-6, dadurch gekennzeichnet, daß die Nukleotidsequenz codierend für ein oder mehrere Proteine ausgewählt aus der Gruppe A oder Teile davon in sense- oder anti-sense-Richtung vorliegt.

10. Vektor, enthaltend ein Nukleinsäuremolekül nach einem oder mehreren der Ansprüche 2-6, dadurch gekennzeichnet, daß die Nukleotidsequenz codierend für ein oder mehrere Proteine ausgewählt aus der Gruppe A teilweise in sense-Richtung und teilweise in anti-sense-Richtung vorliegt.

11. Vektor, enthaltend ein Nukleinsäuremolekül nach einem oder mehreren der Ansprüche 1-6, dadurch gekennzeichnet, daß es mit regulatorischen Elementen

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 99/05536

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 5763252	A	09-06-1998	NONE		
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			EP	0871744 A	21-10-1998
			JP	11514521 T	14-12-1999

VERTRAG ÜBER DIE INTERNATIONALE ZUSAMMENARBEIT AUF DEM GEBIET DES PATENTWESENS

PCT

INTERNATIONALER RECHERCHENBERICHT

(Artikel 18 sowie Regeln 43 und 44 PCT)

Aktenzeichen des Anmelders oder Anwalts 1998/M225PCT	WEITERES VORGEHEN	siehe Mitteilung über die Übermittlung des internationalen Recherchenberichts (Formblatt PCT/ISA/220) sowie, soweit zutreffend, nachstehender Punkt 5
Internationales Aktenzeichen PCT/EP 99/ 05536	Internationales Anmeldedatum *(Tag/Monat/Jahr)* 30/07/1999	(Frühestes) Prioritätsdatum *(Tag/Monat/Jahr)* 31/07/1998
Anmelder HOECHST SCHERING AGREVO GMBH ET AL		

Dieser Internationale Recherchenbericht wurde von der Internationalen Recherchenbehörde erstellt und wird dem Anmelder gemäß Artikel 18 übermittelt. Eine Kopie wird dem Internationalen Büro übermittelt.

Dieser Internationale Recherchenbericht umfaßt insgesamt 5 Blätter.

[X] Darüber hinaus liegt ihm jeweils eine Kopie der in diesem Bericht genannten Unterlagen zum Stand der Technik bei.

1. **Grundlage des Berichts**

   a. Hinsichtlich der **Sprache** ist die Internationale Recherche auf der Grundlage der internationalen Anmeldung in der Sprache durchgeführt worden, in der sie eingereicht wurde, sofern unter diesem Punkt nichts anderes angegeben ist.

   [ ] Die internationale Recherche ist auf der Grundlage einer bei der Behörde eingereichten Übersetzung der internationalen Anmeldung (Regel 23.1 b)) durchgeführt worden.

   b. Hinsichtlich der in der internationalen Anmeldung offenbarten **Nucleotid– und/oder Aminosäuresequenz** ist die Internationale Recherche auf der Grundlage des Sequenzprotokolls durchgeführt worden, das

   [X] in der internationalen Anmeldung in schriftlicher Form enthalten ist.

   [X] zusammen mit der internationalen Anmeldung in computerlesbarer Form eingereicht worden ist.

   [ ] bei der Behörde nachträglich in schriftlicher Form eingereicht worden ist.

   [ ] bei der Behörde nachträglich in computerlesbarer Form eingereicht worden ist.

   [ ] Die Erklärung, daß das nachträglich eingereichte schriftliche Sequenzprotokoll nicht über den Offenbarungsgehalt der internationalen Anmeldung im Anmeldezeitpunkt hinausgeht, wurde vorgelegt.

   [ ] Die Erklärung, daß die in computerlesbarer Form erfaßten Informationen dem schriftlichen Sequenzprotokoll entsprechen, wurde vorgelegt.

2. [X] **Bestimmte Ansprüche haben sich als nicht recherchierbar erwiesen** (siehe Feld I).

3. [ ] **Mangelnde Einheitlichkeit der Erfindung** (siehe Feld II).

4. Hinsichtlich der **Bezeichnung der Erfindung**

   [X] wird der vom Anmelder eingereichte Wortlaut genehmigt.

   [ ] wurde der Wortlaut von der Behörde wie folgt festgesetzt:

5. Hinsichtlich der **Zusammenfassung**

   [X] wird der vom Anmelder eingereichte Wortlaut genehmigt.

   [ ] wurde der Wortlaut nach Regel 38.2b) in der in Feld III angegebenen Fassung von der Behörde festgesetzt. Der Anmelder kann der Behörde innerhalb eines Monats nach dem Datum der Absendung dieses internationalen Recherchenberichts eine Stellungnahme vorlegen.

6. Folgende Abbildung der **Zeichnungen** ist mit der Zusammenfassung zu veröffentlichen: Abb. Nr. –

   [ ] wie vom Anmelder vorgeschlagen

   [ ] weil der Anmelder selbst keine Abbildung vorgeschlagen hat.

   [ ] weil diese Abbildung die Erfindung besser kennzeichnet.

   [ ] keine der Abb.

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Feld I Bemerkungen zu den Ansprüchen, die sich als nicht recherchierbar erwiesen haben (Fortsetzung von Punkt 2 auf Blatt 1)

Gemäß Artikel 17(2)a) wurde aus folgenden Gründen für bestimmte Ansprüche kein Recherchenbericht erstellt:

1. ☐ Ansprüche Nr.
weil sie sich auf Gegenstände beziehen, zu deren Recherche die Behörde nicht verpflichtet ist, nämlich

2. ☒ Ansprüche Nr. 2-4,6,7,9-25
weil sie sich auf Teile der internationalen Anmeldung beziehen, die den vorgeschriebenen Anforderungen so wenig entsprechen, daß eine sinnvolle internationale Recherche nicht durchgeführt werden kann, nämlich
Siehe Zusatzblatt WEITERE ANGABEN PCT/ISA/210

3. ☐ Ansprüche Nr.
weil es sich dabei um abhängige Ansprüche handelt, die nicht entsprechend Satz 2 und 3 der Regel 6.4 a) abgefaßt sind.

Feld II Bemerkungen bei mangelnder Einheitlichkeit der Erfindung (Fortsetzung von Punkt 3 auf Blatt 1)

Die internationale Recherchenbehörde hat festgestellt, daß diese internationale Anmeldung mehrere Erfindungen enthält:

1. ☐ Da der Anmelder alle erforderlichen zusätzlichen Recherchengebühren rechtzeitig entrichtet hat, erstreckt sich dieser internationale Recherchenbericht auf alle recherchierbaren Ansprüche.

2. ☐ Da für alle recherchierbaren Ansprüche die Recherche ohne einen Arbeitsaufwand durchgeführt werden konnte, der eine zusätzliche Recherchengebühr gerechtfertigt hätte, hat die Behörde nicht zur Zahlung einer solchen Gebühr aufgefordert.

3. ☐ Da der Anmelder nur einige der erforderlichen zusätzlichen Recherchengebühren rechtzeitig entrichtet hat, erstreckt sich dieser internationale Recherchenbericht nur auf die Ansprüche, für die Gebühren entrichtet worden sind, nämlich auf die Ansprüche Nr.

4. ☐ Der Anmelder hat die erforderlichen zusätzlichen Recherchengebühren nicht rechtzeitig entrichtet. Der internationale Recherchenbericht beschränkt sich daher auf die in den Ansprüchen zuerst erwähnte Erfindung; diese ist in folgenden Ansprüchen erfaßt:

Bemerkungen hinsichtlich eines Widerspruchs

☐ Die zusätzlichen Gebühren wurden vom Anmelder unter Widerspruch gezahlt.

☐ Die Zahlung zusätzlicher Recherchengebühren erfolgte ohne Widerspruch.

WEITERE ANGABEN PCT/ISA/ 210

Fortsetzung von Feld I.2

Ansprüche Nr.: 2-4,6,7,9-25

Die geltenden Patentansprüche 2-4, 6, 7 und 9-25 beziehen sich auf eine unverhältnismäßig große Zahl möglicher Nukleinsäuremoleküle, Verfahren und Pflanzen von denen sich nur ein kleiner Anteil im Sinne von Art. 6 PCT auf die Beschreibung stützen und/oder als im Sinne von Art.5 PCT in der Patentanmeldung offenbart gelten kann. Im vorliegenden Fall fehlt den Patentansprüchen die entsprechende Stütze und fehlt der Patentanmeldung die nötige Offenbarung in einem solchen Maße, daß eine sinnvolle Recherche über den gesamten erstrebten Schutzbereich unmöglich erscheint. Daher wurde die Recherche auf die Teile der Patentansprüche gerichtet, welche im o.a. Sinne als gestützt und offenbart erscheinen, nämlich die Teile betreffend, die Nukleinsäuren, Verfahren und Pflanzen wie sie in den Ausführungsbeispielen 1-12 angegeben sind, nämlich alpha-Glukosidase kodierende DNA-Sequenzen in sense und antisense Orientierung, gegebenenfalls in Kombination mit DNA-Sequenzen kodierend für lösliche Stärkesynthase I, II, III in sense und antisense Orientierung, sowie Kombinationen mit BE/SSIII, SSI/SSIII und SSII/SSIII in antisense Orientierung.

Der Anmelder wird darauf hingewiesen, daß Patentansprüche, oder Teile von Patentansprüchen, auf Erfindungen, für die kein internationaler Recherchenbericht erstellt wurde, normalerweise nicht Gegenstand einer internationalen vorläufigen Prüfung sein können (Regel 66.1(e) PCT). In seiner Eigenschaft als mit der internationalen vorläufigen Prüfung beauftragte Behörde wird das EPA also in der Regel keine vorläufige Prüfung für Gegenstände durchführen, zu denen keine Recherche vorliegt. Dies gilt auch für den Fall, daß die Patentansprüche nach Erhalt des internationalen Recherchenberichtes geändert wurden (Art. 19 PCT), oder für den Fall, daß der Anmelder im Zuge des Verfahrens gemäß Kapitel II PCT neue Patentanprüche vorlegt.

INTERNATIONALER RECHERCHENBERICHT

Internationales Aktenzeichen
PCT/EP 99/05536

A. KLASSIFIZIERUNG DES ANMELDUNGSGEGENSTANDES
IPK 7 C12N15/55 C12N15/54 C12N15/82 C12N15/11 C12N9/26
C12N5/10 C08B30/00 A01H5/00 A01H5/10 A23L1/0522

Nach der Internationalen Patentklassifikation (IPK) oder nach der nationalen Klassifikation und der IPK

B. RECHERCHIERTE GEBIETE

Recherchierter Mindestprüfstoff (Klassifikationssystem und Klassifikationssymbole)
IPK 7 C12N C08B A01H A23L

Recherchierte aber nicht zum Mindestprüfstoff gehörende Veröffentlichungen, soweit diese unter die recherchierten Gebiete fallen

Während der Internationalen Recherche konsultierte elektronische Datenbank (Name der Datenbank und evtl. verwendete Suchbegriffe)

C. ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie°	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X	SUGIMOTO M. ET AL.: "Molecular cloning and characterization of a cDNA encoding alpha-glucosidase from spinach" PLANT MOLECULAR BIOLOGY, Bd. 33, 1997, Seiten 765-768, XP002130610 in der Anmeldung erwähnt das ganze Dokument	6,7,11, 12,24
X	US 5 763 252 A (TIBBOT BRIAN K ET AL) 9. Juni 1998 (1998-06-09) das ganze Dokument	6,7,11, 12,24
X	WO 97 24448 A (NICKERSON BIOCEM LTD ;TAYLOR MARK ANDREW (GB); DAVIES HOWARD VIVIA) 10. Juli 1997 (1997-07-10) das ganze Dokument	1,3,5-7, 11-25

-/--

[X] Weitere Veröffentlichungen sind der Fortsetzung von Feld C zu entnehmen

[X] Siehe Anhang Patentfamilie

° Besondere Kategorien von angegebenen Veröffentlichungen :
"A" Veröffentlichung, die den allgemeinen Stand der Technik definiert, aber nicht als besonders bedeutsam anzusehen ist
"E" älteres Dokument, das jedoch erst am oder nach dem Internationalen Anmeldedatum veröffentlicht worden ist
"L" Veröffentlichung, die geeignet ist, einen Prioritätsanspruch zweifelhaft erscheinen zu lassen, oder durch die das Veröffentlichungsdatum einer anderen im Recherchenbericht genannten Veröffentlichung belegt werden soll oder die aus einem anderen besonderen Grund angegeben ist (wie ausgeführt)
"O" Veröffentlichung, die sich auf eine mündliche Offenbarung, eine Benutzung, eine Ausstellung oder andere Maßnahmen bezieht
"P" Veröffentlichung, die vor dem Internationalen Anmeldedatum, aber nach dem beanspruchten Prioritätsdatum veröffentlicht worden ist
"T" Spätere Veröffentlichung, die nach dem Internationalen Anmeldedatum oder dem Prioritätsdatum veröffentlicht worden ist und mit der Anmeldung nicht kollidiert, sondern nur zum Verständnis des der Erfindung zugrundeliegenden Prinzips oder der ihr zugrundeliegenden Theorie angegeben ist
"X" Veröffentlichung von besonderer Bedeutung; die beanspruchte Erfindung kann allein aufgrund dieser Veröffentlichung nicht als neu oder auf erfinderischer Tätigkeit beruhend betrachtet werden
"Y" Veröffentlichung von besonderer Bedeutung; die beanspruchte Erfindung kann nicht als auf erfinderischer Tätigkeit beruhend betrachtet werden, wenn die Veröffentlichung mit einer oder mehreren anderen Veröffentlichungen dieser Kategorie in Verbindung gebracht wird und diese Verbindung für einen Fachmann naheliegend ist
"&" Veröffentlichung, die Mitglied derselben Patentfamilie ist

Datum des Abschlusses der Internationalen Recherche	Absendedatum des Internationalen Recherchenberichts
16. Februar 2000	02/03/2000

Name und Postanschrift der Internationalen Recherchenbehörde	Bevollmächtigter Bediensteter
Europäisches Patentamt, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Kania, T

Formblatt PCT/ISA/210 (Blatt 2) (Juli 1992)

INTERNATIONALER RECHERCHENBERICHT

Internationales Aktenzeichen
T/EP 99/05536

C.(Fortsetzung) ALS WESENTLICH [illegible]SEHENE UNTERLAGEN

Kategorie°	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X	WO 94 09144 A (ZENECA LTD) 28. April 1994 (1994-04-28)	22,23
A	siehe das ganze Dokument, insbes. S.8-12; S.43-45; Bsp.4	2-4, 6-21,24, 25
X	WO 95 07355 A (INST GENBIOLOGISCHE FORSCHUNG ;KOSSMANN JENS (DE); VIRGIN IVAR (DE) 16. März 1995 (1995-03-16) in der Anmeldung erwähnt	22,23
A	das ganze Dokument	2-4,6-9, 11-21, 24,25
X	WO 97 11188 A (KOSSMANN JENS ;LORBERTH RUTH (DE); PLANTTEC BIOTECHNOLOGIE GMBH (D) 27. März 1997 (1997-03-27) in der Anmeldung erwähnt	22,23
A	siehe das ganze Dokument; insbes. Bsp.10,11	2-4,6-9, 11-21, 24,25
X	WO 96 15248 A (ABEL GERNOT J ;INST GENBIOLOGISCHE FORSCHUNG (DE); KOSSMANN JENS () 23. Mai 1996 (1996-05-23) in der Anmeldung erwähnt	22,23
A	siehe das ganze Dokument; insbes. S.26/27	2-4, 6-21,24, 25
X	EP 0 779 363 A (NAT STARCH CHEM INVEST) 18. Juni 1997 (1997-06-18) in der Anmeldung erwähnt	22,23
A	das ganze Dokument	6-21,24, 25
X	WO 92 14827 A (INST GENBIOLOGISCHE FORSCHUNG) 3. September 1992 (1992-09-03) in der Anmeldung erwähnt	22,23
A	das ganze Dokument	2-4, 6-21,24, 25
A	WO 97 16554 A (INNES JOHN CENTRE INNOV LTD ;SMITH ALISON MARY (GB); DENYER KAY (G) 9. Mai 1997 (1997-05-09) siehe das ganze Dokument; insbes. Patentansprüche	1-25